RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (4) 1552-1562, 2024
http://doi.org/10.54910/sabrao2024.56.4.21
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT ACTIVITY, ANTIMICROBIAL EVALUATION, AND CYTOTOXICITY EFFECTS OF WILD MEDICINAL PLANTS

N.M. KHEDR[1], A.A. IBRAHIM[2*], M. EL-METWALLY[3], S. ELDAKROORY [1], and M.I. SOLIMAN[4]

[1]Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Mansoura University, Egypt
[2]Botany and Microbiology Department, Faculty of Science, Arish University, Al-Arish, 45511, Egypt
[3]Mycological Research Department, Plant Pathology Research Institute, ARC, Giza, Egypt
[4]Botany Department, Faculty of Science, Mansoura University, Egypt
Corresponding author's emails: amiranasreldeen@Sci.aru.edu.eg, amiranasreldeen@yahoo.com
Email addresses of co-authors: nadamohsen2030@gmail.com, elmetwally2030@gmail.com, eldakrory2030@gmail.com, magdasoliman2030@gmail.com

SUMMARY

Egyptian wild medicinal plants are *Nicotiana glauca* R.C., *Solanum incanum* L., and *Withania somnifera* L. The phytochemicals, antibacterial activity, and cytotoxicity of each species' 1%, 3%, and 5% aqueous extracts attained scrutiny. Five fungal and five bacterial species (*Aspergillus niger*, *A. flavus*, *A. terreus*, *Alternaria alternata*, and *Rhizoctonia solani*) trials used the antimicrobial activity, agar well diffusion method. The *Vicia faba* assay showed the cytotoxicity of these plants at varied doses. *N. glauca* had 1.65% tannins and the most antioxidant activity at 0.32 mg/ml. *N. glauca* had a superior antifungal activity against *A. niger*, *A. terreus*, *R. solani*, *B. cereus*, *P. aeruginosa*, and *R. solanacearum*. At 1%, 3%, and 5% aqueous extracts, *W. somnifera* has the largest chromosomal aberrations, 22.97%, 25.46%, and 40.93%, respectively. In anaphase and telophase, cytotoxicity causes interphase micronucleus, stickiness, disrupted metaphase, lagging, bridge, and diagonal abnormalities. For reducing the pharmaceutical cytotoxicity, this study provided information on wild medicinal plants, revealing *N. glauca* with the greatest phytochemicals, antioxidants, and antibacterial activity with minimum cytotoxicity and abnormalities. Hence, its use can be safe for therapeutic dosages.

Keywords: Wild medicinal plants, *Nicotiana glauca*, *Solanum incanum*, *Withania somnifera*, antimicrobial activity, phytochemical analysis, cytotoxicity

Communicating Editor: Dr. Kamile Ulukapi

Manuscript received: October 17, 2023; Accepted: March 26, 2024.

Citation: Khedr NM, Ibrahim AA, El-Metwally M, Eldakroory S, Soliman MI (2024). Phytochemical analysis, antioxidant activity, antimicrobial evaluation, and cytotoxicity effects of wild medicinal plants. *SABRAO J. Breed. Genet.* 56(4): 1552-1562. http://doi.org/10.54910/sabrao2024.56.4.21.

Key findings: *Nicotiana glauca*, *Solanum incanum*, and *Withania somnifera* are Egyptian wild medicinal herbs with high antioxidant secondary metabolites. *N. glauca* had the most phytochemicals, antioxidant, and antibacterial activity but the lowest cytotoxic effect and abnormality percentage. The findings suggest using *N. glauca* aqueous extracts at 1%, 3%, or 5% for specific medical disorders.

INTRODUCTION

Ancient healing and modern pharmaceutical development depend on medicinal plants (Mengistu *et al*., 2010). About 800 plant species treat 300 physiological and psychological disorders (Sbhatu and Abraha, 2020). Low-toxicity bioactive natural medicines offer therapeutic promise. Plants are rich in phytochemicals and physiologically active substances with pharmacological properties used in traditional medicine for millennia (Nadeem *et al*., 2022). Medical plants contain saponins, flavonoids, tannins, alkaloids, essential oils, and compounds (Prabhavathi *et al*., 2016). Plants employ phytochemicals to adapt and combat viruses and pests. Many medicinal plants have fascinating pharmacological properties and phytoconstituents that may generate safer disease-treating chemicals (Nabi *et al.,* 2022). Pharmacological research increasingly requires medicinal plant bioactive molecule screening (Sorrenti *et al*., 2022). Solanaceae bioactive substances with antibacterial, anticancer, and antioxidant properties can treat numerous diseases (Eissa *et al*., 2023). Solanaceae produces antimicrobial peptides, flavonoids, glycosides, alkaloids, lignans, steroids, sugars, simple phenols, and terpenoids (Ghatak *et al*., 2017). The Solanaceae family is antioxidant, anti-inflammatory, antibacterial, and anticancer. The Solanaceae family comprises medicinal plants, such as *Solanum*, *Atropa*, *Nicotiana*, and *Withania*. Solanaceae contains 80 genera and 3,000 species—the Solanum family includes 1,500 species (Gebhardt, 2016).

Economically important *Solanum* has 50% of the family's species. *Solanum*, the biggest Solanaceae genus, comprises 1250–1700 species. Herbal medicine employs *Solanum* extensively. Solasodine is antioxidant, anticancer, neuroprotective, and hepatoprotective (Deshmukh *et al.,* 2023). *Solanum incanum* phytochemistry revealed alkaloids, flavonoids, saponins, terpenoids, glycosides, and steroids (Sbhatu and Abraha, 2020). *S. incanum* produces tannins, flavonoids, phenols, saponins, alkaloids, glycosides, steroids, and terpenoids. Microorganisms' antibiotic resistance is a global health issue. *S. incanum* inhibits *E. coli*, *S. pyogenes*, *S. aureus*, and *P. aeruginosa*. Foreign plants may be exotic, introduced, or non-native (Ahmed *et al*., 2020).

Nicotiana glauca, or tree tobacco, is an invasive plant spreading fast in Egypt, Saudi Arabia, Croatia, Mexico, the US, South Africa, Morocco, Namibia, and Australia. The 76 *Nicotiana* species are predominantly from North and South America and Australia. *Nicotiana tabacum* L. emerged as the principal tobacco leaf and product source (Drapal *et al*., 2022). Anatabine, anabasine, and nicotine are present in *N. glauca* tissues. Anabasine, an alkaloid like nicotine, is harmful and widespread. Secondary metabolites of *N. glauca* are antibacterial, antiviral, anti-inflammatory, antiallergic, antiasthma, antimalarial, cytotoxic, and anticancer, and may cure neurological illnesses. Traditional healers utilize *N. glauca* preparations for antibacterial, antifungal, antiviral, anti-inflammatory, and cytotoxic effects. *N. glauca* plants are also attractive decorations. The leaves' high larvicidal indolic alkaloids make it a promising bioinsecticide (Al-Harbi *et al*., 2021).

Withania somnifera is a Solanaceae medicinal plant ashwagandha (Kumar *et al*., 2023). The alkaloid content makes *W. Somnifera* a safe dietary supplement. Egyptians, Djiboutians, and Ethiopians treat Alzheimer's, malaria, and bronchitis with *W. somnifera* and other plants. *W. somnifera* phytochemical analysis contains alkaloids, saponins, tannins, sugars, steroidal lactones, phenols, flavonoids, somniferine, somniferinine, withanine, withanolides, and

withananine. The main phytochemicals are withanolides and steroidal lactones. *W. somnifera* kills tumor cells but not healthy human cells. *W. somnifera* is anti-tumoral, cytotoxic, genotoxic, antibacterial, antifungal, antiangiogenic, anti-depressive, and anti-metastatic. *W. somnifera* leaf can treat microbial diseases due to its antibacterial capabilities. Antioxidant-rich *W. somnifera* roots treat immune systems, infertility, and cardiovascular illnesses according to their highest antioxidant activity (Kelm *et al*., 2020). This study examined the following: 1) the primary constituents and phytochemical analysis for three Solanaceae species (*Nicotiana glauca, Solanum incanum*, and *Withania somnifera*), 2) antioxidant activity of these wild medicinal plants, 3) the antimicrobial potential of aqueous extracts of these plants against different fungal and bacterial species, and 4) the cytotoxic effect of the plant aqueous extract using the chromosomal aberration assay.

MATERIALS AND METHODS

Plant materials

Three species from the family Solanaceae (*Nicotiana glauca*, *Solanum incanum*, and *Withania somnifera*), collected from their natural habitats, received identification by Prof. Ibrahim A. Mashaly, Professor of Plant Flora and Ecology, Botany Department, Faculty of Science, Mansoura University. The locations, life forms, and habitat types are available in Table 1.

Phytochemical analyses

Drying and grinding to powder of shoot systems from the studied wild medicinal plants ensued. About 10 g from each plant sustained phytochemical analysis, as follows:

Total ash

Over a burning flame, a silica crucible containing 3 g powder from each studied plant acquired heating. The charred material's heating between 600 and 500 °C took 6 h in a muffle furnace. After cooling, its weight obtained on ash-free filter paper.

Crude fiber

Powder from each sample's digestion had H_2SO_4 and NaOH, then continued its burning in a muffle furnace at 550 °C for 4 h to extract the crude fiber.

Total lipids

For 16 h, extracting 10 g of powder from each sample used a Soxhlet apparatus and petroleum ether (b.p. 60 °C–80 °C), with each extract dried by evaporating it over anhydrous Na_2SO_4. The lipid content determination resulted from drying the residue at 80 °C for ten minutes, letting it cool, and then weighing it (Soliman *et al.*, 2022).

Table 1. Location and habitat type of the selected wild plants collected from Egypt.

Taxon	Family	District	Governorate	Life span	Life form	Habitat type	Location (GPS) Data	
							Latitude (N)	Longitude (E)
Nicotiana glauca R.C. Graham	Solanaceae	Burg El-Arab	Alexandria	Perennial	Phanerophyte	Road side	30°59'30.85"	29°43'14.22"
Solanum incanum L.	Solanaceae	Burg El-Arab	Alexandria	Perennial	Chamaephyte	Barley fields	30°55'0"	29°32'0"
Withania somnifera L.	Solanaceae	Mansoura	EL-Dakahlia	Perennial	Chamaephyte	Cultivated land	31°02'10.93"	31°22'50.48"

Total protein

The protein content derivative came from measuring the nitrogen content of each sample using the micro-Kjeldahl method.

Total carbohydrates

The powder from around 0.2 g per sample reached hydrolysis with 5 cm^3 of HCl (2.5 N) in a boiling water bath for 3 h and then cooled at room temperature. After adding Na_2CO_3 to the mixture and waiting for the ebullition to stop, the volume increased to 100 cm^3, continued centrifugation to separate the supernatant. The supernatant's cooking in a boiling water bath contained 4 cm^3 of anthrone reagent for 8 min, with the color displaying green to dark green at 630 nm.

Total phenols

The Folin Ciocalteu (FC) technique helped quantify total phenols in peel crude extracts. The Sigma gallic acid (GA, 5%) developed the curve. Diluting extracts to 10 l occurred upon pouring into test tubes. Then, adding 0.5 ml of FC reagent had 4 min allowed to pass. After incubating each sample for 2 h at room temperature in the dark with 1 mL of Na_2CO_3 (7.5%, W/V), the measured absorbance was at 760 nm (Abd-Ellatif *et al*., 2022).

Total flavonoids

Total flavonoid measurement used the $AlCl_3$ colorimetry. After 5 min at room temperature, adding 10 ml of each extract to 100 ml of 5% sodium nitrite (W/V) continued. Next, 1 ml of sodium hydroxide NaOH (1M) and enough distilled water to reach 5 ml ensued after 5 min incubation with 100 ml of $AlCl_3$ (10%, W/V). After mixing well for 15 min, the recorded absorbance was at 510 nm against a blank. The total flavonoid concentration measured was in CE (BOH Chemicals Ltd., Poole, England) per ml of crude extract.

Total alkaloids

At room temperature, 1 g of the material bore 4 h exposure to 50 mL of 10% acetic acid in ethanol. Filtered material concentration resulted from a water bath. Slowly pouring ammonium hydroxide over the extracted mixture precipitated it. After settling the solution, the residue washing used diluted ammonium hydroxide, then filtered and dried to constant weight.

Saponins content

Each 20 g of powder added to a pot of 20% watered-down ethanol reached cooking for 4 h while stirring. Filtered over a 90 °C water bath helped reduce the mixture to 40 ml. After adding 1 ml of concentrate to the separator funnel, 2 ml of diethyl ether incorporation continued stirring vigorously. Following water separation, adding 4 ml of n-butanol proceeded washing twice with 2 ml of 5% aqueous sodium chloride solution. A water bath heated the residual solution. After evaporation, the samples were oven-dried to a uniform weight to assess saponin concentration.

Tannins content

A vanillin-hydrochloride test determined tannin levels. Values for the extracted plant samples' tannin contents had expressions as grams of tannic acid equivalents per one hundred grams of dry plant. Tannin content determination had the data fitted to a standard curve constructed from tannic acid ($y = 0.0009x$; $r^2 = 0.955$).

Terpenoids

About 0.5 g of the extract combined with about 2 mL of $CHCl_3$ had a thin layer of concentrated H_2SO_4 (about 3 mL) added. The presence of terpenoids is indicative of a characteristic red color.

Steroids

A few drops of Ac_2O addition to the filtrate ensued after treating the extract with $CHCl_3$. Then, boiling and cooling the solution confirmed the existence of phytosterols; at this point, a brown ring forms at the junction.

Antioxidant activity: Free-radical scavenging activity using DPPH assay

The extracts' ability to remove the "stable" free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique had the DPPH (0.3 mm) in ethanol combined with 2.0 ml test materials. The reaction mixture remained at room temperature in a dark room for 30 min. At 517 nm, a Perkin Elmer Lambda 265 spectrophotometer assessed the color change from deep violet to pale yellow. The decline in absorbance measured free radical removal, which helped calculate antioxidant activity, as shown in the following equation:

$$\text{DPPH scavenging capacity (\%)} = \frac{(A\ control - A\ sample)}{A\ control}\ x\ 100$$

Where, A control represents the absorbance of the control and A sample is the absorbance of the test sample.

Antimicrobial activity

The antibacterial activity of the studied wild plants (5 g crude extracts in 100 ml H_2O) employed well diffusion. The assay used five strains of fungi (*Aspergillus niger, A. flavus, A. terreus, Alternaria alternata,* and *Rhizoctonia solani*) and five bacterial species (*Bacillus subtilis*,*B. cereus*, *Pseudomonas aeruginosa*, *Ralstonia solanacearum*, and *Micrococcus luteus*), evenly dispersed across the Mueller Hinton agar (MHA) and broth (Difco Laboratories, Detroit, USA) for growth. Similarly, fungal cultures on potato dextrose agar (PDA) continued at 27 °C for seven days. One hundred microliters of each plant extract continued placement in Petri dishes. The control antibiotics used were tetracycline as standard. The studies in triplicate reached incubation at 37 °C for 24 h for bacterial strains and 25 °C for 48 h for fungal pathogens. Clear inhibition zones surrounding wells suggested antibacterial action. The inhibitory zone diameter (mm) of crudes and antibiotics was compared (Soliman *et al.*, 2022, 2023).

Chromosome aberration assay

The National Gene Bank, Ministry of Agriculture, and Land Reclamation provided the *Vicia faba* L. (Misr 1) seeds. The seeds germinated in Petri plates between two layers of cotton after submerging in distilled water at 26 °C for 24 h until roots measured 1.5–2.0 cm in length. *Vicia faba* seeds treatment comprised three concentrations (1%, 3%, and 5%) from each aqueous wild plant extract and then storing *V. faba* root tips in a refrigerator for at least 48 h after being fixed in a glacial acetic acid/ethanol with the ratio of 1:3 (Carney's solution). Hydrolysis continued in 1N HCl at 60 °C for 6-8 min, followed by a 5-min wash in distilled water. Then, preparing a slide had the root tips rinsed in water and staining them with aceto-orcein stain (Soliman *et al*., 2023) for 2–4 h. One drop of 45% acetic acid on a clean slide received the darkly stained root tips squished under a covered glass to disperse the cells. Recording normal and abnormal cells at various mitotic phases engaged an electric microscope (Olympus CX 40). Mitotic index (MI), phase index (PI), and total abnormality percentage served to assess cytotoxicity across cell-cycle phases.

Statistical analysis

The study used means ± standard deviations (SD) to show the data. The Statistical Package for Social Sciences (SPSS) 16 helped probe the data, with the analysis of variance (ANOVA) reviewing the data (IBM SPSS Statistics, Chicago, IL, USA). Duncan's test revealed how different methods stacked against each other. Statistical differences were significant at the $p \leq 0.05$ level. T-tests statistically analyzed estimates of the difference between varying concentrations from each sample and the control for cytotoxicity assay using *V. faba* test plants (Soliman *et al.*, 2017).

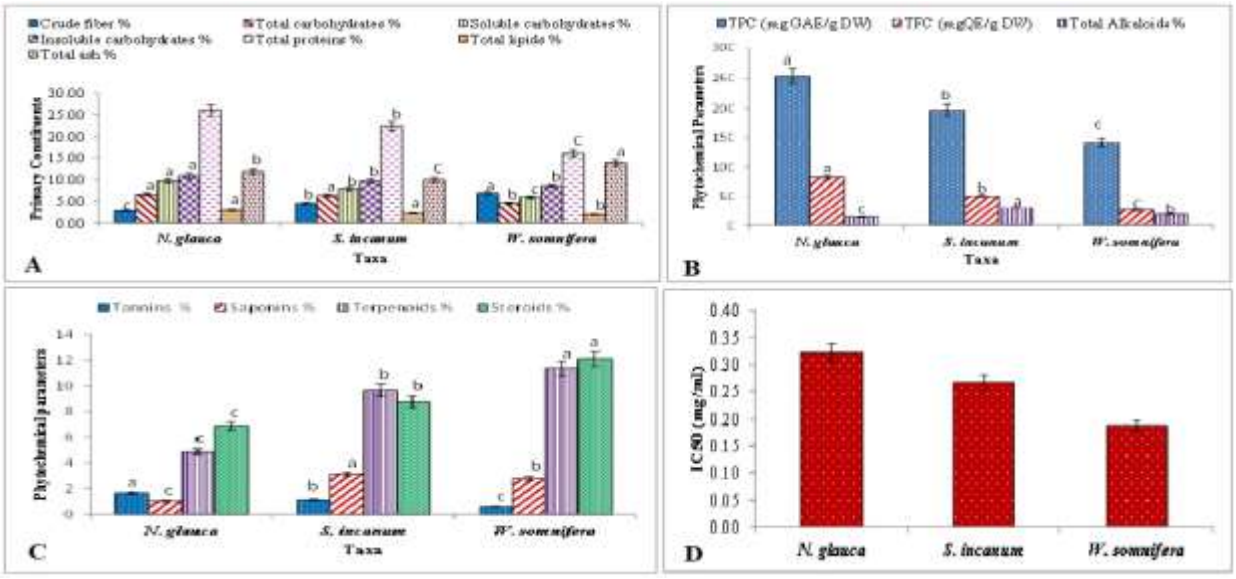

Figure 1. A: Primary constituents; B & C: showing different phytochemical parameter; D: Antioxidant activity using radical scavenging activity (IC50) for the studied wild medicinal plants. Different letters indicate significant differences between different treatments at $P \leq 0.05$.

RESULTS

Primary constituents and photochemical analysis

Figure 1A shows the primary ingredients. *W. somnifera* had the highest crude fiber concentration at 6.84%, followed by *S. incanum* at 4.47%. The highest total carbohydrate concentration was 6.58% in *N. glauca* and 4.51% in *W. somnifera*. The greatest soluble and insoluble carbohydrate concentrations were 9.76% and 10.87%, respectively, in *N. glauca*. Total protein content was highest in *N. glauca* (26.03%) and *S. incanum* (22.42%). *N. glauca* had the most significant total lipid content at 2.98%, and *W. somnifera* had the lowest at 2.02%. *W. somnifera* had the maximum total ash content at 13.91%, while *S. incanum* had the lowest at 9.93%.

Figure 1B showed that *N. glauca* had the highest total phenolic and total flavonoids at 252.98 mg GAE/g DW and 80.65 mgQE/g DW, respectively, and *W. somnifera* had the lowest at 140.59 mg GAE/g DW and 26.19 mgQE/g DW, respectively. Total alkaloids were highest in *S. incanum* (30.43%) and lowest in *N. glauca* (4.54%).

The examined medicinal plants' tannins, saponins, terpenoids, and steroids appear in Figure 1C. The highest tannin values were 1.65% for *N. glauca* and 1.12% for *S. incanum*. Saponin content ranged from 3.11% in *S. incanum* to 1.03% in *N. glauca*. *Withania somnifera* had the maximum terpenoids and steroid concentrations, 11.34% and 12.09%, whereas *N. glauca* had the lowest at 4.87% and 6.87%, respectively.

Antioxidant activity using DPPH assay

Figure 1D illustrates the antioxidant activity using the DPPH assay. The highest radical scavenging activity (IC50) was 0.32 ± 0.001 mg/ml in *N. glauca,* followed by 0.19 mg/ml in *S. incanum,* and the lowest radical scavenging activity (IC50) was 0.19 mg/ml found in *W. somnifera*.

Antimicrobial activity

Table 2 and Figure 2 show the agar well diffusion method's antimicrobial activity of the

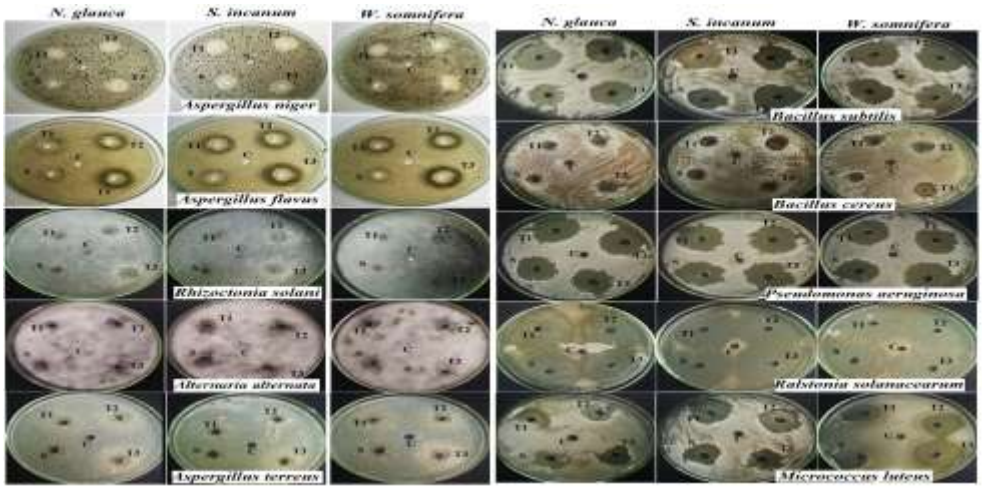

Figure 2. Antifungal and antibacterial activities of the studied medicinal plants aqueous extracts; C: negative control; S: standard; T1: aqueous extract 1%; T2: aqueous extract 3%; T3: aqueous extract 5 %.

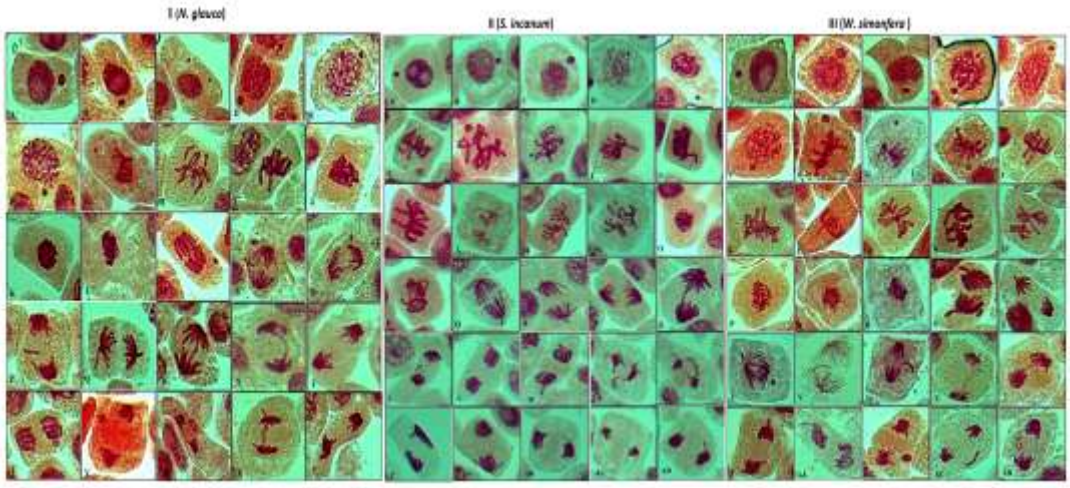

Figure 3. I-III: Different types of chromosome aberrations for three concentrations (1%, 3%, & 5%) of *N. glauca*, *S. incanum,* and *W. somnifera* aqoueus extracts. I: From (A–D) Micronucleus at interphase stage, (E, F) Micronucleus at prophase, (G) Micronucleus at metaphase, (H, I) Non-congression at metaphase, (J–L) Stickiness at metaphase, (M–O) Micronucleus at anaphase, (P) Laggard at anaphase, (Q) Disturbed at anaphase, (R, S) Bridge at anaphase, (T) Diagonal at anaphase, (U, V) Micronucleus at telophase, (W, X) Bridge at telophase, (Y) Disturbed at telophase; II: From (A–C) Micronucleus at interphase stage, (D–F) Micronucleus at prophase, (F–H) Micronucleus at metaphase, (I–K) Disturbed at metaphase, (L–N) Two groups at metaphase, (O, P) Stickiness at metaphase, (Q, R) Disturbed at anaphase, (S) Diagonal at anaphase, (T, U) Bridge at anaphase, (V–X) Disturbed at telophase, (Y) Bridge at telophase, (Z, AA) Diagonal at telophase, (AB, AD) Late separation at telophase; III: From (A–D) Micronucleus at interphase stage, (E, F) Micronucleus at prophase, (G–I) Micronucleus at metaphase, (K–M) Disturbed at metaphase, (N, O) Two groups at metaphase, (P–R) Stickiness at metaphase, (S, T) Disturbed at anaphase, (U) Micronucleus at anaphase, (V, W) Laggard at anaphase, (X, Y) Bridge at telophase, (Z, AA) Disturbed at telophase, (AB) Micronucleus at telophase, (AC,AD) Laggard at telophase, (X = 1000).

Table 2. Antimicrobial activity of the aqueous extracts for the studied wild medicinal plants against different species of fungi and bacteria.

Taxa	Aqueous conc.	Mean of inhibition zone (in mm)									
		Fungi					Bacteria				
		A. niger	*A. flavus*	*A. terreus*	*A. alternata*	*R. solani*	*B. subtilis*	*B. cereus*	*P. aeruginosa*	*R. solanacearum*	*M. luteus*
N. glauca	1 %	7±0.23ab	7±0.15ab	7.5±0.25a	7.5± 0.25a	7±0.18ab	20±0.51c	7±0.18e	24±0.68b	32±0.69a	16±0.43d
	3 %	10±0.43a	9±0.19b	10±0.27a	8±0.26b	8.5±0.17b	25±0.61c	9±0.42e	28±0.68b	32±0.71a	17±0.53d
	5 %	13±0.34a	11±0.37bc	11.5±0.39b	10.5±0.36c	12.5±0.38ab	28±0.57c	13±0.32e	30±0.69b	34±0.72a	19±0.43d
S. incanum	1 %	11±0.32b	12±0.35a	7±1.7d	10.5±0.28c	6.5±0.08de	22±0.31c	8±0.23e	23±0.35b	30±0.56a	15±0.29d
	3 %	11.5±0.42b	14±0.46a	8.5±0.21c	11.5±0.35b	8.5±0.19c	26±0.54b	9±0.32e	25±0.51c	32±0.58a	21±0.49d
	5 %	12.5±0.23b	14.5±0.36a	9.5±0.19d	12.5±0.23b	10.5±0.22c	28±0.67	12±0.34	27±0.66	34±0.71	23±0.56
W. somnifera	1 %	8±-0.13c	9±0.15b	7.5±0.12d	9.5±0.16a	7.5±0.12d	26±0.47b	7±0.15e	23±0.43c	28±0.53a	20±0.37d
	3 %	10.5±0.21b	11.5±0.23a	9.5±0.18d	10±0.2c	8.5±0.17e	29±0.48b	9±0.36e	25±0.46c	30±0.59a	22±0.41d
	5 %	12.5±0.23b	13.5±0.28a	11.5±0.17d	12±0.22c	11.5±0.17d	31±0.51b	12±0.31e	28±0.46c	33±0.54a	25±0.41d

* Different letters indicate significant differences between different treatments at $P \leq 0.05$.

Table 3. Mitotic index, normal and abnormal phase indices, total abnormalities in non-dividing and dividing cells after treating *Vicia faba* root tips with different concentrations of aqueous extracts from three wild medicinal plant extracts.

Treatment		MI %	Phase index % (PI)								Total abnormal % (Tab)	
			% Prophase		% Metaphase		% Anaphase		% Telophase		Interphase	Mitosis
Samples	Conc.		mitotic	Abn.	mitotic	Abn.	mitotic	Abn.	mitotic	Abn.		
Control		10.70± 0.45	22.76	0.00	69.88	16.77	0.51	2.47	6.75	1.56	0.03±0.02	20.81±2.09
N. glauca	1 %	15.25 s±0.86	29.76	0.60	24.94	4.66	12.68	3.48	32.62	2.04	0.55 s±0.05	10.78 ns ±0.67
	3%	20.05 ns ±0.93	41.86	2.05	25.69	3.80	12.7	2.53	19.75	5.45	0.29 s±0.01	13.83 ns ±0.54
	5%	10.89 s±0.63	31.62	1.51	37.89	9.99	12.94	1.98	17.55	4.23	0.64 s±0.03	17.71 ns±0.78
S. incanum	1 %	16.41 s±1.01	41.69	0.07	29.52	5.11	18.62	1.54	10.17	6.93	0.12 s±0.02	13.65 ns ±0.56
	3 %	19.35 ns±1.06	20.87	3.05	24.25	3.90	15.36	0.24	39.52	11.61	3.05 ns±0.03	18.80 ns±1.09
	5 %	12.34 s ±0.74	23.29	0.15	30.34	7.18	21.91	5.34	24.46	8.08	0.16 s±0.01	20.75 s±0.98
W. somnifera	1 %	12.08 s±0.88	28.19	1.06	25.82	6.23	18.94	6.00	27.05	9.68	0.36 s±0.01	22.97 s±1.02
	3 %	9.14 ns±0.64	19.17	0.42	38.80	9.50	15.22	5.30	26.81	10.24	0.42 s±0.01	25.46 s±1.09
	5 %	10.18 s±0.53	25.21	0.00	32.95	12.02	13.38	5.64	28.46	23.27	0.39 s±0.02	40.93 s±1.87

researched taxa's aqueous extracts against various fungi and bacteria. *N. glauca* had a robust antifungal efficacy against *A. niger* (inhibition zone 13 mm for 5%) and *A. terreus* (10 and 11.5 mm for 3% and 5%, respectively). *N. glauca* had the optimum antifungal activity against *R. solani*, with a 12.5-mm inhibition zone of 5%. *Solanum incanum* had the best antifungal activity against *A. flavus* and *A. alternata*: 3% and 5%, with inhibition zones 14 and 14.5 mm and 11.5 and 12.5 mm, respectively (Figure 2). *N. glauca* had the highest antibacterial activity against *B. cereus* (9 and 13 mm for 3% and 5%), *P. aeruginosa* (28 and 30 mm for 3% and 5%), and *R. solanacearum* (32 and 34 mm for 3% and 5%). *W. somnifera* had the maximum antibacterial activity against *B. subtilis* and *M. leteus,* with inhibition zones of 29 and 31 mm for 3% and 5%, respectively (Figure 2).

Cytotoxicity assay

Table 3 and Figure 3 showed the cytotoxicity of each medicinal plant at 1%, 3%, and 5%. Table 3 displays MI, PI, and total abnormalities (Tab). *N. glauca* had the most significant rise in MI (20.05%), compared with the control (10.70%), followed by *S. incanum* (19.35%) and *W. somnifera* (3.4%), with 9.14%.

The *N. glauca* (3%) had the ultimate significant mitotic index in prophase (41.86%) and *W. somnifera* (3%) in metaphase (38.80%). *S. incanum* (5% and 3%) had the extreme mitotic index in anaphase and telophase at 21.91% and 39.52%, respectively. The most significant increase in anomalies in prophase was 3.05% for *S. incanum* (3%) and in metaphase, anaphase, and telophase for *W. somnifera* at 5% and 1%, respectively, compared with the control. The highest significant increase in total anomalies was 40.93, 25.46, and 22.97 in *W. somnifera* at 5%, 3%, and 1%, respectively. In comparison, *N. glauca,* at 1%, 3%, and 5%, had the lowest total abnormalities of 10.78%, 13.83%, and 17.71%, respectively.

In Figure 3, chromosome abnormalities appeared for three concentrations of *N. glauca*, *S. incanum,* and *W. somnifera* aqueous extracts. All medicinal plant doses showed an interphase micronucleus. Micronucleus at prophase was evident in all plant concentrations except *W. somnifera* (5%). For all medicinal taxa, metaphase aberrations included disturbed, non-congression, oblique, sticky, and micronucleus. All studied taxa had disturbed late separation, diagonal, bridge, and micronucleus chromosomal abnormalities at anaphase. All taxa had micronucleus, disrupted, late separation, bridges, and diagonal chromosomal abnormalities at telophase.

DISCUSSION

According to phytochemical studies, the plants comprised 15% and physiological studies at 6% (Thomford *et al*., 2018). Many active ingredients in medicinal herbs fight Gram-positive and Gram-negative bacteria. From 1981 to 2019, 162 novel antimicrobials became licensed, with 94% as plant-based. *N. glauca* contains most total phenol, flavonoids, and alkaloids at 252.98 mg GAE/g DW, 80.65 mgQE/g DW, and 14.54%, respectively. Al-Nema and Abdullah (2023) and Eissa *et al.* (2023) found that water, soil microorganism exposure, pH, and nutrients affect secondary metabolite accumulation.

N. glauca contains the most tannins, and leaf chloroplasts biosynthesize and accumulate flavonoids, with aerial tissues containing the most flavonoids because sunlight promotes synthesis (Pollastri and Tattini, 2011). *W. somnifera* phytochemical research identified steroidal chemicals, alkaloids, phenolic compounds, saponins with additional acyl groups, and withanolides with glucose at carbon 27. Singh *et al*. (2023) identified the ultimate steroids (12.09%) and terpenoids (11.34%) in *W. somnifera*. *N. glauca* showed the maximum antioxidant activity at 0.32 mg/ml, finding that tobacco's phenolic components donate H to DPPH free radicals to create DPPH-.H. Zhong *et al.* (2020) observed that TPC assay favorably linked with ABTS, DPPH, and FRAP antioxidant activities.

A positive and significant linear relationship emerged between Solanaceae species' antioxidant activity and total

phenolics. Phenols and flavonoids give *Solanum nigrum* and other Solanaceae antioxidant action (Jimoh *et al*., 2010). Many research stated polyphenolics, flavonoids, steroidal saponins, triterpenoids saponin, and steroidal glycoside have antimicrobial effects. Aqueous *N. glauca* extracts demonstrated the best antibacterial activity against *A. niger*, *A. terrus*, *R. solani*, *B. cereus*, *P. aeruginosa*, and *R. solanacearum*. Antimicrobial peptides can kill bacteria by binding to DNA in the cytoplasm after crossing the membrane. Shakirin *et al.* (2012) found Canarium species latex antioxidant, antibacterial, anti-inflammatory, and blood sugar regulator. Lupeol is the most antioxidant, antibacterial, antihyperglycemic, and anticancer (Vats and Gupta, 2017). Certain phytochemicals are highly effective against *Escherichia coli* and *Staphylococcus aureus*. *V. faba* root tips have been utilized for cytogenetic investigations for 50 years since they assess physical and chemical clastogenicity (Soliman *et al*., 2023). Anomalies in chromosomal structure are called aberrations. DNA damage, synthesis interference, and replication errors can induce inherited chromosomal abnormalities (Soliman *et al*., 2017). The three *N. glauca* concentrations (1%, 3%, and 5%) showed the lowest anomalies (10.78%, 13.83%, and 17.71%). *W. somnifera* had the most anomalies at 22.97%, 25.46%, and 40.93% for 1%, 3%, and 5%.Micronuclei, stickiness, laggards, late separations, and bridges were chromosome defects. Interphase micronuclei may indicate spindle fiber distortion (Soliman *et al*., 2017). DNA depolymerization and nucleoprotein breakdown can produce chromosomal condensation and stickiness (Ma *et al*., 2012). Cell adhesion increased with concentration. Lagging chromosomes result from not migrating to either pole (Soliman *et al*., 2017; 2023).

CONCLUSIONS

Nicotiana glauca has the highest antioxidant activity and phytochemical constituents, with low chromosomal abnormalities, making it a drug candidate. More clinical trials are necessary to assess this chemical's medicinal advantages and safety.

REFERENCES

Abd-Ellatif S, Ibrahim AA, Safhi FA, Abdel Razik ES, Kabeil SSA, Aloufi S, Alyamani AA, Basuoni MM, ALshamrani SM, Elshafie HS (2022). Green synthesized of *Thymus vulgaris* Chitosan nanoparticles induce relative WRKY-genes expression in *Solanum lycopersicum* against *Fusarium solani*, the causal agent of root rot disease. *Plants* 11, no. 22: 3129. https://doi.org/10.3390/plants11223129.

Ahmed D, Ammar E, Svenning J, El-Beheiry M, Shaltout K (2020). Wild plant species in Egyptian gardens of the Nile Region: Conservation viewpoint. *Egypt. J. Bot.* 60(3): 719–732. doi: 10.21608/ejbo.2020.21253.1414.

Al-Harbi NA, Al Attar NM, Hikal DM, Mohamed SE, Abdel Latef AAH, Ibrahim AA, Abdein MA (2021). Evaluation of insecticidal effects of plants essential oils extracted from basil, black seeds and lavender against *Sitophilus oryzae*. *Plants.* 10:829. doi: 10.3390/plants10050829.

Al-Nema QS, Abdullah RM (2023). Propagation protocol of the medicinal plant - Aloe vera using tissue culture. *SABRAO J. Breed. Genet*. 55(1): 254- 259. http://doi.org/10.54910/sabrao2023.55.1.23.

Deshmukh V, Deshpande J, Wani M (2023). Elicitation based enhancement of solasodine production in in-vitro cultures of different *Solanum* species. *J. Environ. Biol.* 44:167–174.

Drapal M, Enfissi EM, Fraser PD (2022). The chemotype core collection of genus *Nicotiana*. *Plant J.* 110(5): 1516–1528. https://doi.org/10.1111/tpj.15745.

Eissa N, Ibrahim AA, Soliman M (2023). Comparative studies among some cultivated species and their wild relatives in Egypt using phytochemical screening, nutritive value, antioxidant activity and GC-MS analysis. *Catrina: Int. J. Environ. Sci.* 28(1): 1–19. doi: 10.21608/cat.2023.202700.1167.

Gebhardt C (2016). The historical role of species from the Solanaceae plant family in genetic research. *Theor. Appl. Genet*. 2281–2294. https://doi.org/10.1007/s00122-016-2804-1.

Ghatak A, Chaturvedi P, Paul P, Agrawal GK, Rakwal R, Kim ST, Weckwerth W, Gupta R (2017).

Proteomics survey of Solanaceae family: Current status and challenges ahead. *J. Proteomics.*169:41–57. https://doi.org/10.1016/j.jprot.2017.05.016.

Jimoh FO, Adedapo AA, Olayan AJ (2010). Comparison of the nutritional value and biological activities of the acetone methanol and water extracts of the leaves of *Solanum nigrum* and *Leonotis leonorus*. *Food Chem. Toxicol.* 48(3): 964–971.

Kelm NQ, Straughn AR, Kakar SS (2020). Withaferin A attenuates ovarian cancer-induced cardiac cachexia. *Plos One 15*: e0236680.

Kumar S, Mathew SO, Aharwal RP, Tulli HS, Mohan CD, Sethi G, Ahn K, Webber K, Sandhu SS, Bishayee A (2023). Withaferin A: A pleiotropic anticancer agent from the Indian medicinal plant *Withania somnifera* (L.) Dunal. *Pharmaceuticals* 16(2): 160. https://doi.org/10.3390/ph16020160.

Ma X, Wang X, Liu Y (2012). Cytotoxicity and geno-toxicity evaluation of urban surface waters using freshwater luminescent bacteria Vibrio-qinghaiensis sp.-Q67 and *Vicia faba* root tip. *J Environ Sci* (China) 24(10): 1861–1866.

Mengistu (2010). An Ethnobotanical Study of Medicinal Plants in Seru Wereda, Arsi Zone of Oromia Region. Addis Ababa University, Addis Ababa, Ethiopia.

Nabi M, Tabassum N, Ganai BA (2022). *Skimmia anquetilia* N.P. Taylor and Airy Shaw (Rutaceae): A critical appraisal of its ethnobotanical and pharmacological activities. *Front. Plant Sci.* 13:930687. https://doi.org/10.3389/fpls.2022.930687.

Nadeem A, Shahzad, Ahmed B, Muntean T, Waseem M, Tabassum A (2022). Phytochemical profiling of antimicrobial and potential antioxidant plant: Nepeta cataria. *Front. Plant Sci.* 13: 969316. https://doi.org/10.3389/fpls.2022.969316.

Pollastri S, Tattini M (2011). Flavonols: Old compound for old roles. *Ann. Bot*. 108(7): 1225–1233.

Prabhavathi RM, Prasad MP, Jayaramu M (2016). Studies on qualitative and quantitative phytochemical analysis of *Cissus quadrangularis*. *Adv. Appl. Sci. Res*. 7: 11–17.

Sbhatu DB, Abraha HB (2020). Preliminary antimicrobial profile of *Solanum incanum* L.: A common medicinal plant. *Evid. Based Complement. Alternat. Med*. 2020:3647065. doi: 10.1155/2020/3647065. PMID: 32063983; PMCID: PMC6996673.

Shakirin FH, Azlan A, Ismail A, Amom Z, Cheng Y (2012). Protective effect of pulp oil extracted from *Canarium odontophyllum* Miq. fruit on blood lipids, lipid peroxidation, and antioxidant status in healthy rabbits. *Oxidative Med. Cell. Longev.* 2012:840973.

Singh KB, Hahm ER, Kim SH, Singh SV (2023). Withaferin A inhibits fatty acid synthesis in rat mammary tumors. *Cancer Prev. Res.* 16: 5–16.

Soliman MI, Ibrahim AA, El-Metwally MA, Eshak DA (2017). Cytotoxicity effects of biological control and antioxidants using *Vicia faba* chromosomal aberration assay. *J. Environ. Sci. Technol.* 10: 230–237. doi: 10.3923/jest.2017.230.237.

Soliman MI, Mohammed NS, EL-Sherbeny G, Safhi FA, ALshamrani SM, Alyamani AA, Alharthi B, Qahl SH, Al Kashgry NAT, Abd-Ellatif S, Ibrahim AA (2023). Antibacterial, antioxidant activities, GC-Mass characterization, and cyto/genotoxicity effect of green synthesis of silver nanoparticles using latex of *Cynanchum acutum* L. *Plants.* 12:172. https://doi.org/10.3390/plants12010172.

Soliman SA, Hafez EE, Al-Kolaibe AMG, Abdel Razik ES, Abd-Ellatif S, Ibrahim AA, Kabeil SSA, Elshafie HS (2022). Biochemical characterization, antifungal activity, and relative gene expression of two *Mentha* essential oils controlling *Fusarium oxysporum*, the causal agent of *Lycopersicon esculentum* root rot. *Plants* 11, no. 2: 189. https://doi.org/10.3390/plants11020189.

Sorrenti V, Burò I, Consoli V, Vanella L (2022). Recent advances in health benefits of bioactive compounds from food wastes and by-products: Biochemical aspects. *Int. J. Mol. Sci.* 24(3):2019. https://doi.org/10.3390/ijms24032019.

Thomford NE, Senthebane DA, Rowe A, Munro D, Seele P, Maroyi A, Dzobo K (2018). Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. *Int. J. Mol. Sci.* 19: 1578.

Vats S, Gupta T (2017). Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of *Moringa oleifera* Lam. from Rajasthan, India. *Physiol. Mol. Biol. Plants. 23*:239–248.

Zhong B, Robinson NA, Warner RD, Barrow CJ, Dunshea FR, Suleria HAR (2020). LC-ESI-QTOF-MS/MS Characterization of seaweed phenolics and their antioxidant potential. *Mar. Drugs*. 18(6): 331.