



Phytochemical Analysis, Antioxidant and Antimicrobial Activities of Emergent *Cyperus laevigatus* L

Yasser A. El-Amier ^{a,*}, and Abdelkrem M.B. Abdalla ^b

^aBotany Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt

^bFaculty of Nursing, Zawia University, Zawia, Libya



Abstract

Biological control is receiving increasing attention as an alternative means of disease control, especially where disease resistance or chemical control are not available. The aim of this study was to investigate the phytochemical analysis of emergent *Cyperus laevigatus* L and assess its antioxidant and antimicrobial activities. Methanol extract's preliminary phytochemical screening revealed differences in active secondary chemicals. For the rhizome, stem, and leaves, respectively, phenolics were found to be 267.06, 79.48, and 96.46 mg of gallic acid equivalent/g dry extract, flavonoids were 90.62, 27.78, and 34.47 mg of catechin equivalent/g dry extract; and tannins were 32.83, 12.46, and 23.35 mg of gallic acid equivalent/g dry extract. With an IC₅₀ value of 30, 40, 42, and 51.76 mg/L for the rhizome, stem, and leaf, respectively, the DPPH antioxidant activity of the *C. laevigatus* extracted components showed that the rhizome extract is the most potent. The current findings indicate that *C. laevigatus* rhizome extract had superior antibacterial activity against the Gram-positive bacterial strains, while methanolic extract had a greater zone of inhibition against *Pseudomonas aeruginosa* and *Escherichia coli* (25.8 and 22.2 mm, respectively) (*Staphylococcus aureus* and *Bacillus subtilis*). The separation of pure components from the *C. laevigatus* extracts will be the focus of future research. Important biological results also suggested that more research on this plant may make it possible to find new medications made from natural sources.

Keywords: *Cyperus laevigatus*; Halophytes; Antibacterial; Antioxidant; Secondary metabolites

1. Introduction

Globally, there are wild plants that make up nature's biodiversity. About 60 to 80% of the world's inhabitants rely on plant medicines, and they offer a variety of food and herbal remedies [1, 2]. With a few oases here and there, Egypt's environment is mostly desert. The Gulf of Suez, Aqaba, the Mediterranean Sea, and the Red Sea all have extensive coastlines. A variety of plants and animals inhabit each geographical area, each one adapted to its ecosystem [3,4]. Salt swamplands, sandbanks, panes, sandy fertile zones, and lake shorelines are the five habitat types that may be found along Egypt's deltaic Mediterranean Sea coast [5]. Additionally, a large number of tasty species and/or species rich in nutrients can be found in Egypt's natural vegetation along the Mediterranean coast, making them valuable

natural resources to produce food, fodder, and medicines [6].

Numerous native medicinal herbs are utilized both as food additions and for medical purposes [7]. The therapeutic value of these plants is due to their bioactive phytochemical constituents, which have been utilized for ages in traditional medicine to treat a wide range of ailments. The most active phytochemicals found in plants are glycosides, tannins, flavonoids, flavones, and alkaloids. These organic substances served as the foundation for contemporary medications as they are used today [8, 9]. Antioxidative phytochemicals and resistance to antimicrobial agents have received increasing attention worldwide for their potential role in preventing the risk of human diseases [10, 11].

*Corresponding author e-mail: yasran@mans.edu.eg (Y.E-A.).

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With 109 genera and almost 5,500 species, the Cyperaceae (sedge family) is the third-biggest family of monocots and the largest family of monocotyledons [12, 13]. With more than 600 species worldwide, particularly in pantropical and tropical regions, the *Cyperus* genus is the biggest in this family [14,15]. *Cyperus* species are broadly used as wildflowers in traditional medicines as an anthelmintic, carminative, diuretic, analgesic, stimulant, astringent, and colic remedy [16,17]. The biological activity of *Cyperus* spp. revealed that the plant as a drug has numerous therapeutic actions, such as antimicrobial, hepatoprotective, antiinflammatory, antimalarial, gastroprotective, and antidiabetic [15,18-22]. Previous phytochemical studies on *Cyperus* plants indicate the presence of aurones [23], quinones [24], sesquiterpenes, steroids [25], essential oils [26], nitrogenous compounds [27], phenolics [28], and flavonoids [29, 15].

Cyperus laevigatus L. (Family: Cyperaceae, syns. *C. mucronatus*), also called borbeit or Sa'ed in Egypt, is a tufted perennial sedge with crowded stems (up to 60 cm tall) or single stems on a long creeping rhizome [30,14]. *C. laevigatus* thrives in wet areas, especially in shallow brackish water, moist alluvial and sandy soils, near wells and springs, on the edges of salt marshes, and in alkaline habitats. In Egypt, *C. laevigatus* is commonly distributed in the Nile Delta, Oases, Mediterranean coast, Red Sea coast, Gebel Elba, and Sinai [14]. The aerial parts of the *C. laevigatus* alcoholic extract show tannins, phenolics, flavonoids, and glycosides in a preliminary phytochemical analysis. The plant alcohol extract has also been linked to a few other pharmacological effects, including anti-inflammatory, antioxidant, antidiabetic, and cytotoxic actions [28].

Alcoholic extract of *C. laevigatus* was reported to have antiinflammatory and antidiabetic activities, but phytochemical analyses were not determined except for phenolic constituents [28]. Moreover, little is known about the preliminary phytochemical analysis and biological activity of *C. laevigatus*, as well as allelopathic activity. To determine the antioxidant and antibacterial properties of a crude extract derived from *C. laevigatus*, which is used to cure a variety of ailments, the goal of this study was to screen for phytochemicals in the rhizome and leaves of *C. laevigatus* in Egypt.

2. Materials and Methods

2.1. Preparation of plant material

Cyperus laevigatus fresh plant components (rhizome, stem, and leaves) were collected during the flowering stage from sand flats in Egypt's northern Nile Delta (31°29'28.08"N, 31°23'46.90"E). According to Täckholm [31] and Boulos [32], the examined species were identified. The gathered plants were crushed into a fine powder and stored in a polypropylene container for later use after being dried for 21 days in the shade at room temperature.

After being washed, the samples were given time to air dry. 10 g of dried plant material was put into a conical flask that had a capacity of 250 mL and could hold 150 mL of methanol. The mixture was then placed in a water bath shaker (Memmert WB14, Schwabach, Germany), where it was continually stirred for two hours at room temperature. Whatman filter papers were used to filter the mixture (no. 1, 125 mm, Cat. No. 1001 125, Germany). The final plant extract concentrations were studied, and the extracts were kept at 4 °C for storage [33].

2.2. Phytochemical analysis

The active components were removed using a method like hot infusions used in conventional therapy. 20 grams of each dried plant part were combined with 200 mL of deionized water and agitated for 30 minutes in a water bath system that was heated to 70 °C. Filtration of the produced extract and storage of the filtrate at 4 °C. Quantitative estimates were made of the total amounts of flavonoid, tannin, and phenolic compounds in the aqueous extract of *C. laevigatus* aerial and underground parts.

2.2.1. Total Phenolic Contents

The total phenolic content was assessed using the Folin-Ciocalteu method developed by Wolfe *et al.* [34], using gallic acid as a reference. The measured total phenolic content in the aqueous extract of *C. laevigatus* was quantified as equivalents in milligrams of gallic acid/dried plant extract in grams using a standard curve ($y = 0.0074x$, $r^2 = 0.969$).

2.2.2. Total Flavonoid Contents

Zhishen *et al.* [35] used catechin as a reference and a colorimetric assay with aluminium chloride to

calculate the total quantity of flavonoids present. The value of the total flavonoid components was estimated as milligram equivalents of catechin per dried plant extract in grams using a standard curve ($y = 0.0031x$, $r^2 = 0.991$).

2.2.3. Total Tannin Contents

According to Burlingame [36] and Aberoumand [37], the vanillin-hydrochloride test was used to determine the total amount of tannins, and the comparable amounts of tannic acid in grams per dried plant per 100 g were used to express the values of the anticipated samples.

2.3. Antioxidant activities

2.3.1. DPPH radical scavenging capacity estimation

According to Miguel's assay [38], the radical scavenging capacity of *C. laevigatus* extract was assessed using the 2, 2-diphenyl-1-picryl-hydrazil (DPPH) radical. Simply put, 2 ml of plant extracts at concentrations ranging from 100 to 1000 mg/l were combined with 2 ml of 150 M DPPH and then left in the dark at 40 °C for 30 minutes. All samples were examined in triplicate; the absorbance was at 520 nm using a Milton Roy Spectronic 21D UV-Visible Spectrophotometer (USA), and the IC₅₀ values were calculated using an exponential curve.

$$\% \text{ Of DPPH scavenging} = [1 - (A_{\text{sample}} / A_{\text{control}})] \times 100$$

2.4. Antibacterial potential

2.4.1. Tested bacteria

The antimicrobial potential of *C. laevigatus* extract was estimated for eight pathogenic bacteria including 5 gram-negative bacteria (*Klebsiella pneumonia* (ATCC10031), *Listeria monocytogenes* (ATCC19116), *Escherichia coli* (ATCC10536), *Salmonella typhi* (ATCC25566) and *Pseudomonas aeruginosa* (ATCC9027)) and 3 gram-positive bacteria (*Streptococcus epidermis* (EMCC1353), *Staphylococcus aureus* (ATCC6538) and *Bacillus subtilis* (DMS1088)).

2.4.2. Disc diffusion assay

Discs were loaded over the plates containing the tested strains after being submerged in the examined extracts for a period of 18 to 24 hours at 37 °C. The diameter of the filter paper discs (6 mm) was

subtracted from the predicted zone of growth inhibition [39].

3. Results and Discussion

3.1. Phytochemical constituents

Herbal medicines' therapeutic advantages are based on the plant's chemistry. Understanding the chemical makeup of plants aids in understanding their potential medical uses. The primary metabolic pathways are used by the plant cell to produce a large number of secondary plant metabolites [40]. Among plant secondary metabolites, phenolic compounds were shown to have considerable antiinflammatory, antioxidant, antihyperglycemic, immunomodulatory, and anticancer activity [41-44]. The present study demonstrated that the parts of *C. laevigatus* extract are filled with phenolics (267.06, 79.48, and 96.46 mg gallic acid equivalent/g dry extract), flavonoids (90.62, 27.78, and 34.47 mg catechin equivalent/g dry extract), and tannins (32.83, 12.46, and 23.35 mg gallic acid equivalent/g dry extract) for the rhizome, stem, and leaves, respectively. These substantial variations may result from ecological variables, species, plant age, organ genetic variables, and the condition of secondary metabolism in various growth environments [45].

Our research on the antioxidant potentials and functions of flavonoids and phenolics has been published in recent works. In this respect, they both significantly stimulate and inhibit oxidative reactions [46]. Additionally, they can break radical chains, which have been demonstrated to have a potent antioxidant impact. In addition to flavonoids, tannins, and phenolics, there are widespread and substantial plant phytochemicals. The second-most prevalent polyphenol, tannins, primarily serve as defensive chemicals that shield plants from pests and other abiotic stresses like drought, heat, and intense UV radiation [47]. Moreover, tannins have strong antibacterial and antioxidant properties [41, 48]. Elshamy *et al.* [28] state that a phytochemical study of aboveground biomass revealed the presence of *C. laevigatus* phenolics as well as its anti-inflammatory and antidiabetic capabilities. Quinones, flavonoids, sesquiterpenes, steroids, and essential oils were among the secondary metabolites described from *Cyperus* sp. [17, 25, 29, 49].

Table 1. The phytochemical analysis of *Cyperus laevigatus* parts.

Samples	Phytochemical Analysis		
	Phenolics	Flavonoids	Tannins
Rhizome	267.06±3.65	90.62±3.41	32.83±1.08
Stem	79.48±2.87	27.78±1.86	12.46±0.93
Leaves	96.46±2.66	34.47±1.52	23.35±0.75

Phenolics Content "mg gallic acid/1 gm dry extract", Flavonoids Content "mg catechine/1 gm dry extract", Tannins Content "mg tannic acid/1 gm dry extract"

3.2. Biological characteristics of the plant extracts

3.2.1. Antioxidant activity - DPPH assay

In vitro assays, 2, 2 diphenyl-1-picrylhydrazyl radical scavenging assay was used to determine the antioxidant capacity of *Cyperus laevigatus* methanolic extract. Table 2 indicated an increasing trend in DPPH radical scavenging activity with an increase in the concentration of extract. The results are reported in Table 2 using half maximum inhibitory concentration (IC₅₀) values to express the scavenging activities of plant extracts and the standard on the DPPH radical. An increased ability to scavenge DPPH radicals is shown by a decreased IC₅₀ value. It was further demonstrated that the

extract from the rhizome, with an IC₅₀ of 30.40 mg/L, has the greatest antioxidant scavenging activity when compared to ascorbic acid (IC₅₀= 14.16 mg/mL), followed by the stem and leaves (IC₅₀ = 42.40 and 51.76 mg/mL, respectively) as shown in Table 2. Because of the sample's enhanced ability to trick free radicals in the solution due to the presence of phenolics, the results generally match those obtained from phytochemical analyses. According to El-Amier *et al.* [50], Elshamy *et al.* [28], Al-Rowaily *et al.* [51], and Abd-ElGawad *et al.* [52], the results of *Cyperus laevigatus* were in contract with the results of the present study.

Table 2. Radical scavenging activity (%), and IC₅₀ values (mg/L) at various concentrations of the methanol extracted from *Cyperus laevigatus* and the standard ascorbic acid by DPPH assay.

Concentrations (mg/mL)	% of DPPH scavenging		
	Rhizome	Stem	Leaves
50	73.83±2.84	57.25±2.73	50.03±1.97
40	67.64±2.05	46.06±1.40	38.52±2.05
30	52.81±1.60	39.23±1.19	26.86±1.53
20	31.68±0.96	28.10±0.85	16.54±0.97
10	22.74±0.69	17.16±0.52	8.40±0.43
5	12.33±0.37	8.51±0.26	6.88±0.21
IC ₅₀ (mg/mL)	30.40	42.40	51.76
LSD _{0.05}		0.0004***	
	Ascorbic acid		
20	62.30±1.13		
15	53.56±0.97		
10	42.52±0.77		
5	35.53±0.27		
2.5	7.68±0.02		
1	1.89±0.02		
IC ₅₀ (mg/mL)	14.16		
LSD _{0.05}	1.82***		

Values are means ± standard error (n=3). IC₅₀: the antioxidant concentration capable of diminishing 50% of the used DPPH radical.

Alternatively, the standard curves, which were drawn as shown in Figure 1, represented the relationship between the sample concentration and the percentage of scavenging activity in all samples.

All samples exhibiting proportional relationships showed an increase in the % activity when the sample concentration was increased [53].

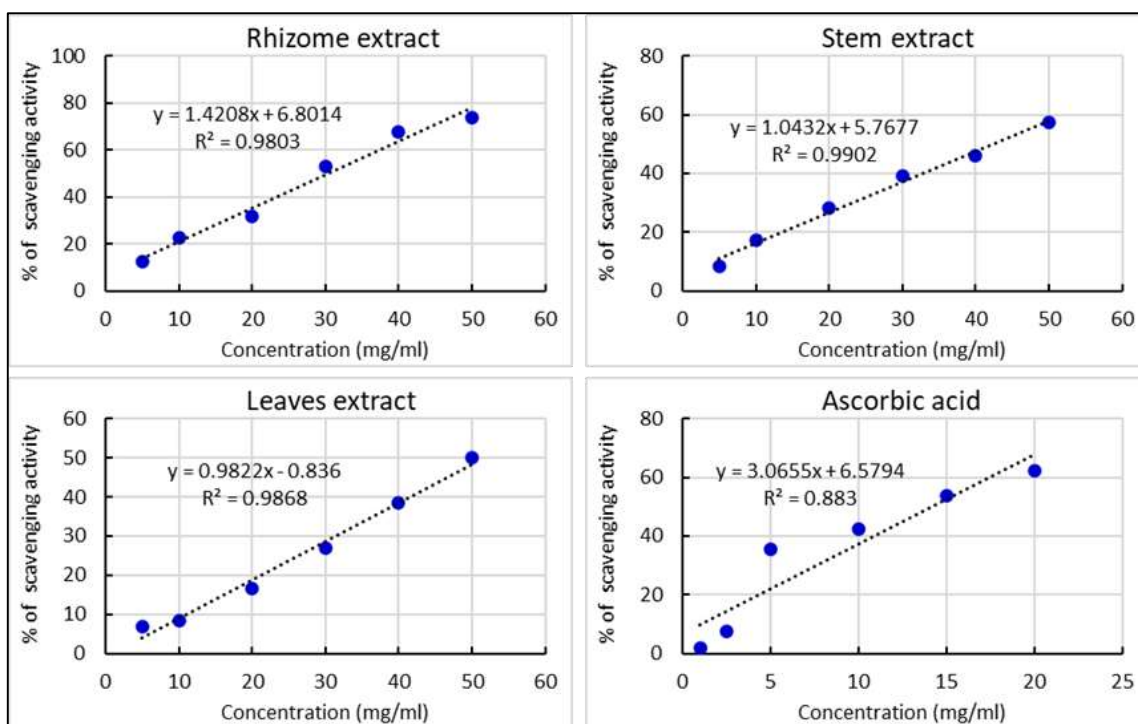


Fig. 1. The standard curves of % of scavenging activities versus sample concentrations.

On the other hand, the antioxidant strength of bioactive substances is often affected by how well reactive oxygen species like phenolics, fatty acids, terpenes, oxygenated hydrocarbons, or carbohydrates can get rid of or stabilize free radicals [54,55]. In terms of antioxidant activity in this investigation, *Cyperus laevigatus* extract surpassed other wild plant extracts from other nations. Many studies have shown that the ability of plants to act as antioxidants is directly related to the amount of bioactive chemicals they have, especially phenolic elements like flavonoids, phenolic acids, ascorbic acids, and carotenoids [56]. Our research indicates that this plant contains phenols, flavonoids, and tannins, among other non-volatile compounds.

3.2.2. Antibacterial Activity

In this study, the antibacterial effects of a methanolic extract of *Cyperus laevigatus* rhizome, stem, and leaf at a concentration of 10 mg/L against five Gram-negative and three Gram-positive bacterial strains were assessed using the previously discussed agar disc diffusion assay method [41]. The outcomes showed that a certain plant's methanolic extract successfully suppresses the growth of harmful bacteria with varied potencies (Table 3). Rhizome

extracts demonstrated more antibacterial activity than stem and leaf extracts against *P. aeruginosa* and *E. coli* (25.8 and 22.2 mm, respectively), whereas all three extracts tested (rhizome, stem, and leaf) demonstrated equivalent activity against *K. pneumoniae* (Table 3). All the studied extracts did not affect *S. typhi* and *L. monocytogenes*, though. Against the Gram-positive strains (*S. aureus* and *B. subtilis*), however, rhizome extract showed higher antibacterial activity, while stem extract demonstrated higher antibacterial activity. Only the *Cyperus laevigatus* rhizome extract, however, demonstrated any antibacterial activity on *S. epidermis*; the other extracts lacked such activity. According to their level of activity, the examined extracts can be grouped as follows: rhizome > stem > leaf.

Different levels of activity were present in the common antibiotics, such as ampicillin, penicillin, gentamicin, and chloramphenicol. While *L. monocytogenes* was completely resistant to ampicillin and chloramphenicol, as well as *K. pneumoniae*, *S. typhi*, *S. epidermis* and *B. subtilis* were totally resistant to ampicillin and penicillin, although they were both completely susceptible to the other (Table 3).

Table 3. Antibacterial activity of methanol extract from the aerial parts of *Cyperus laevigatus* and some selected reference antibiotics at a concentration of 10 mg/ml.

Tested microorganism	<i>Cyperus laevigatus</i> extracts (10 mg/ml)			Standard antibiotic (10 mg/ml)				LSD _{0.05}
	Rhizome	Stem	Leaf	AMP	PEN	GEN	CHL	
Gram-negative bacteria								
<i>Klebsiella pneumoniae</i>	13.8±0.4	12.8±0.2	11.9±0.3	17±0.2	ND	22±0.8	31±0.5	1.051***
<i>Listeria monocytogenes</i>	11.7±0.2	ND	ND	ND	8±0.3	21±0.9	ND	0.461***
<i>Escherichia coli</i>	22.2±0.3	14.2±0.3	9.1±0.2	11±0.3	16±0.5	19±0.8	21±0.7	0.547***
<i>Salmonella typhi</i>	11.2±0.2	ND	ND	ND	12±0.7	22±0.1	20±0.2	0.165***
<i>Pseudomonas aeruginosa</i>	25.8±0.5	14.1±0.2	7.7±0.1	10±0.4	8±0.3	1±0.7	23±0.7	3.739***
Gram-positive bacteria								
<i>Streptococcus epidermis</i>	9.7±0.1	ND	ND	ND	ND	29±0.5	27±0.5	0.316***
<i>Staphylococcus aureus</i>	21.9±0.3	21.7±0.2	5.6±0.2	13±0.5	18±0.7	23±0.8	25±0.9	3.684***
<i>Bacillus subtilis</i>	20.3±0.3	10.8±0.1	6.7±0.2	ND	ND	9±0.4	16±0.7	1.765***

AMP: Ampicillin, PCN: Penicillin, GEN: Gentamicin, CHL: Chloramphenicol. value is the diameter of the inhibition zone (mm) as an average of three replications ± standard error. Significant variation at a probability level of 0.05 (Duncan's test). LSD: least significant difference. *** P < 0.001. ND is referred to as the not detected value "inactive antimicrobial agent".

A preliminary phytochemical study reveals that the alcoholic extract of the emerging plant *C. laevigatus* contains phenolics, tannins, flavonoids, and glycosides. Additionally, the plant alcohol extract's pharmacological effects, including its anti-inflammatory, antibacterial, antioxidant, antidiabetic, and cytotoxic properties, were briefly discussed [28]. According to our research, this plant contains non-volatile substances such as phenols, flavonoids, and tannins. Numerous prior studies on various plant components of various *Cyperus* species demonstrate their powerful antibacterial properties against both Gram-positive and negative bacteria [57–59]. However, they disagreed with Sultan *et al.* [46] on *Senecio glaucus* and Hafaz *et al.* [61] on *Rumex* spp. Abdel-Sattar *et al.* [60] discovered that the methanol extracts of *Plectranthus asirensis* and *Lavandula pubescens* were active against the same tested bacteria strain as well as Salama *et al.* [55] in *Rumex* spp. These outcomes were comparable to those of Joshi *et al.* [62] investigation of *Ocimum sanctum*, *Origanum majorana*, *Cinnamomum zeylanicum*, and *Xanthoxylum armatum*. These differences in the antibacterial latent potency may be related to

environmental factors that affected the constituents of the extract, such as soil type and climate [63].

4. Conclusions.

In comparison to the standards, the results of this study showed that organ extracts from *C. laevigatus* showed promising biological activities, such as antioxidant and antibacterial agents. Particularly, when compared to other plant tissues, rhizome extract displayed the highest antioxidant activity. As opposed to reference standard antibiotics, the methanol extract of several *C. laevigatus* parts demonstrated significant antibacterial activity against Gram-negative species. The exceptional concentration of phenolics, flavonoids, and tannins in the *C. laevigatus* extracts, as well as the significant biological outcomes, suggested that additional research on this plant might be done to find novel medications made from natural sources.

5. Conflicts of interest

"There are no conflicts to declare".

6. Acknowledgments

"None"

7. References

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