Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

*Article*
**Volume 14, Issue 3, 2025, 150**
https://doi.org/10.33263/LIANBS143.150

# Exploring the Pharmacological Potential of *Cyperus rotundus* and *Citrullus colocynthis* Resin: Insights from Behavioral, Anti-inflammatory, and Microbial Studies

**Anis Ben Ali** [1,2,*], **Atef Chouikh** [1,3]

[1] Laboratory Biology, Environment and Health (LBEH), El Oued University, Algeria
[2] El Oued University, Faculty of Natural Science and Life, Cellular and Molecular Biology Department, El Oued, Algeria
[3] El Oued University, Faculty of Natural Science and Life, Biology Department, El Oued, Algeria
* Correspondence: benali-anis@univ-eloued.dz;

**Received: 7.05.2024; Accepted: 6.10.2024; Published: 6.09.2025**

**Abstract:** This study aimed to investigate the potential anti-depressant, anti-inflammatory, and antimicrobial properties of resins extracted from *Cyperus rotundus* tubers and *Citrullus colocynthis* fruits. Experimental assays included the forced swim test (FST), tail suspension test (TST), in vitro bovine serum albumin (BSA) denaturation assay, and evaluation of antimicrobial activity against selected microbial strains. An acute toxicity assessment was conducted to evaluate safety. In the FST, *C. colocynthis* fruit resin significantly reduced immobility time at 40 mg/kg, while *C. rotundus* tuber resin increased immobility time. Both resins showed significant differences compared to the saline control ($p < 0.05$). In the TST, all resin-treated groups exhibited reduced immobility time compared to saline control ($p < 0.001$), with *C. colocynthis* resin demonstrating superiority. The in vitro BSA denaturation assay showed superior anti-inflammatory activity of *C. rotundus* resin compared to *C. colocynthis* resin. Antimicrobial assays revealed both resins' efficacy against selected microorganisms, with higher concentrations exhibiting greater inhibition. *C. rotundus* resin showed higher activity against *Candida albicans.* Our findings suggest potential anti-depressant, anti-inflammatory, and antimicrobial properties of *C. rotundus* and *C. colocynthis* resins, with implications for further pharmacological investigations. These natural products could be explored as novel therapeutic agents for various conditions, though further research is warranted to elucidate their mechanisms and potential clinical applications.

**Keywords:** *Cyperus rotundus*; *Citrullus colocynthis*; resin; antidepressant; anti-inflammatory; antimicrobial.



## 1. Introduction

Depression is a widespread psychiatric condition typified by enduring symptoms of low mood, feelings of despair, and diminished interest or enjoyment in previously rewarding activities [1]. Depression constitutes a considerable global health challenge, impacting millions of people globally and contributing significantly to disability burden and impairment in daily functioning [2]. Despite the availability of various pharmacological treatments, including antidepressant medications, a considerable proportion of patients do not achieve

adequate relief from their symptoms [3], highlighting the need for continued research and development of novel therapeutic approaches.

In recent years, there has been increasing curiosity in investigating the potential antidepressant properties of natural products, particularly those derived from medicinal plants [4]. Traditional herbal remedies have long been used in various cultures to alleviate symptoms of depression and other mood disorders [5]. Consequently, scientific investigations aimed at elucidating these botanical extracts' pharmacological mechanisms and efficacy have gained momentum.

*Cyperus rotundus* and *Citrullus colocynthis* are two plant species known for their traditional medicinal properties [6-8]. Indigenous to regions such as southern Algeria, these plants have been historically utilized for their purported therapeutic effects in treating a range of ailments, including inflammatory conditions and gastrointestinal disorders [9,10].

The present study focuses on evaluating the potential antidepressant-like activity of extracts obtained from the tubers of *C. rotundus* and the fruits of *C. colocynth is* using established animal models of depression. Additionally, the study investigates the anti-inflammatory properties of these extracts using the bovine serum albumin denaturation method. Furthermore, the antimicrobial and antifungal activities of the plant resins are assessed to explore their potential broader pharmacological effects.

The use of animal models such as the Forced Swim Test (FST) and Tail Suspension Test (TST) allows for the systematic evaluation of depressive-like behavior and the screening of potential antidepressant compounds [11,12]. These tests are based on the principle of inducing acute stress in animals and assessing their behavioral responses, which parallel certain aspects of human depression [13]. Additionally, the assessment of anti-inflammatory, antimicrobial, and antifungal activities provides valuable insights into the broader pharmacological profile of the plant extracts.

Overall, the findings of this study hold promise for the development of novel botanical-based therapies for depression and related mood disorders. By elucidating the pharmacological mechanisms underlying the observed effects, this research contributes to the growing body of evidence supporting the therapeutic potential of natural products in mental health care. Furthermore, the exploration of additional pharmacological activities underscores the multifaceted nature of medicinal plants and their potential utility in treating various health conditions beyond depression alone.

## 2. Materials and Methods

### *2.1. Ethics statement.*

The experimental procedures were ethically approved by the Ethics Committee of the Faculty of Life and Natural Sciences, El Oued University, under permits No. 04/2022-2023, dated 20/12/2023.

The animals were treated in accordance with established experimental procedures and protocols outlined in the European Communities Council Directive 2010/63/EU of 22 September 2010.

### *2.2. Chemicals and reagents.*

All chemicals utilized were of analytical grade and acquired from Sigma-Aldrich, Missouri, USA.

*2.3. Plant material.*

In September 2022, subterranean tubers of *C. rotundus* and fruits of *C. colocynthis* were collected from the El Oued province situated in southeastern Algeria. Professor Chouikh Atef verified the botanical identity of the plant specimens. The tubers and fruits were meticulously cleaned using running cold water to eliminate any extraneous matter. Subsequently, they were subjected to a drying period of seven days under dark conditions, following which they were finely ground into a coarse powder and stored for future utilization.

*2.4. Extraction procedure of resin.*

The process of extracting resin from plant materials, as described by Ben Ali et al. [14], involved immersing 100 g of dried powder in a 0.4 L aqueous basic solution (pH = 10) and an antioxidant system for 24 h at room temperature. The resulting infusion was then filtered under pressure until all the solvent was filtered out. Following primary maceration, the residual plant matter underwent secondary maceration in 0.4 L of 97% ethanol, maintaining a pH of 10 and incorporating the identical antioxidant system. The solution was filtered through filter paper, and the resultant material was then dried in an oven set at 50°C, yielding the resin derived from the plant material.

*2.5. Animals.*

Male Wistar rats with an average weight of 160 ± 20 g were obtained from the Pasteur Institute of Algeria. Animals were housed under controlled environmental conditions with a temperature of 25 ± 1°C, a 12-hour light/dark cycle, and approximately 40% relative humidity. Standard laboratory rodent chow, providing a balanced diet of proteins, lipids, carbohydrates, vitamins, and minerals, was provided ad libitum throughout the 15-day experimental period. All animal procedures adhered to established guidelines and protocols outlined in the European Communities Council Directive 2010/63/EU of 22 September 2010 for the protection of animals used for scientific purposes.

*2.6. Acute toxicity assessment.*

The acute toxicity of the resins was evaluated using a modified version of Lorke's method [15-17]. Twenty (20) male Wistar rats (average weight: 160 ± 20 g) were randomly divided into five groups (n = 4/group) following overnight fasting (12 h). Each group received a single intraperitoneal injection of a designated resin dose (control, 40 mg/kg, or 80 mg/kg body weight) prepared in a suitable vehicle. A control group received an intraperitoneal injection of physiological saline only. All animals were continuously monitored for behavioral changes and mortality over a 24-hour period following injection. Subsequently, the surviving animals were observed for a further 10 days.

*2.7. Antidepressant-like action evaluation.*

2.7.1. Forced swim test (FST).

The Forced Swim Test (FST) is a commonly used laboratory assay to assess depressive-like behavior in animals, typically rodents. Originally described by Porsolt et al. [18], the FST involves subjecting animals to a short-term, inescapable stressor, such as being

placed in a cylindrical container filled with water. The immobility period, where the animal ceases to struggle and floats, is considered indicative of behavioral despair, a characteristic associated with depression-like states.

In this study, rats were utilized as the experimental subjects. The cylindrical container used for the test had dimensions of 30 cm in height and 20 cm in diameter, filled with water maintained at a temperature of 26°C up to a level of 15 cm. To increase the test's sensitivity, a 10-minute pre-test session was conducted 24 hours before the main experiment, aimed at inducing a state of hopelessness in the animals.

On the day of the experiment, the mice were pretreated with either extracts from *C. rotundus* tuber resin and *C. colocynthis* fruit resin at doses of 20 or 40 mg/kg or with sulpiride at a dose of 10 mg/kg. These substances were administered 30 minutes prior to subjecting the mice to the FST. Following the pretreatment, each mouse was individually placed in the water-filled cylinder and forced to swim for 6 minutes.It was hypothesized that substances capable of exerting anti-depressant effects would reduce the duration of immobility observed in the FST compared to control animals.

2.7.2. Tail suspension test.

The TST is a behavioral despair test based on the principle that rodents subjected to the inescapable stress of tail suspension exhibit an immobile posture as a measure of behavioral despair [19]. Antidepressant-like drugs are expected to reduce the duration of immobility in this test. In the present study, male Wistar rats were individually suspended by their tail using a retort stand for a total of 6 minutes. The first minute served as an acclimatization period, followed by 5 minutes of observation to record the immobile time [20].

*2.8. In vitro BSA denaturation method.*

The anti-inflammatory activity of the resin was assessed using bovine serum albumin denaturation (BSA) following the method outlined by Chouikh et al. [21]. Briefly, 0.05 mL of various concentrations of the extracts and the reference drug Diclofenac sodium were mixed with 0.45 mL of bovine serum albumin (1% w/v). The mixture was then incubated at 37°C for 20 minutes and subsequently heated at 57°C for 3 minutes. Following cooling, 2.5 mL of phosphate-buffered saline (PBS, pH 6.4) was added, and the absorbance was measured at 416 nm. The percentage of inhibition of protein denaturation was calculated using the formula:

$$\text{Inhibition}\% = 100 - [100 - (\frac{(A1-A0)}{A1}) \times 100] \qquad (1)$$

Where A0 represents the absorbance in the absence of extract, and A1 represents the absorbance in the presence of extract.

*2.9. Antimicrobial activity assessment.*

The antimicrobial and antifungal activities of *C. rotundus* tuber resin and *C. colocynthis* fruit resin were investigated using the disc diffusion method described previously [22].

Bacterial strains included*Staphylococcusaureus* ATCC 25932 (Gram-positive) as the positive control, and *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853,

and *Klebsiella pneumoniae* ATCC 13883 (Gram-negative) as negative controls. The antifungal strain utilized was *Candida albicans* ATCC 14053.

For experimentation, Petri dishes containing Mueller-Hinton agar (for bacteria) or Sabouraud Dextrose Agar (for fungus) were inoculated with the respective test organisms. Sterile filter paper discs impregnated with specific concentrations of the resin extracts were then placed onto the agar surface. Subsequently, the plates were incubated at 37°C for 24 hours (for bacteria) or 48 hours (for fungus). Following the incubation period, the diameter of the clear zone of inhibition surrounding each disc was measured in millimeters (mm) to evaluate the antimicrobial and antifungal activities.

*2.10. Data analysis.*

The results are presented as mean values accompanied by the standard error of the mean (SEM). Statistical analysis was conducted employing the Student's T test via SPSS Statistics software (version 23.0), with significance set at $p < 0.05$.

## 3. Results and Discussion

In this study, we performed different experimental assays, including the forced swim test and the tail suspension test, as these tests are commonly used in preclinical research to evaluate the effectiveness of potential antidepressant drugs. In addition, the study uses an in vitro bovine serum albumin denaturation assay to explore the anti-inflammatory properties of the resin. Furthermore, the study includes an evaluation of the antimicrobial activity of the resin using appropriate microbial strains. The aim of this study is to determine whether the resin exhibits any inhibitory effects against bacterial or fungal pathogens, indicating its potential as an antimicrobial agent.

*3.1. Acute toxicity assessment.*

During the trial period, no fatalities were recorded in the acute toxicity testing across all administered doses. Moreover, there were no indications of behavioral toxicity, such as diarrhea, drooling, lacrimation, defecation, urination, hyperactivity, aggression, piloerection, convulsions, or tremors. Additionally, the absence of convulsions was noted throughout the observation period.

*3.2. Antidepressant-like action evaluation.*

3.2.1. Forced swim test.

The immobility time in the saline group was estimated at 144 ± 26.66 s. A significant alteration in immobility time was observed relative to the saline control group when administering *C. colocynthis* fruit resin at a concentration of 40 mg/kg, resulting in an average immobility time of 82 ± 11.26 seconds. Conversely, administration of *C. rotundus* tuber resin at the same concentration increased immobility time, with an average of 196±0.01 seconds compared to the saline-treated group. Both disparities demonstrated statistical significance ($P < 0.05$). Moreover, neither resin at a concentration of 20 mg/kg substantially impacted immobility time. Notably, treatment with sulpiride at a concentration of 10 mg/kg demonstrated a significant and efficacious reduction in fixation time, with an average of 30.24 ± 19.69 seconds ($P < 0.001$) (Figure 1).

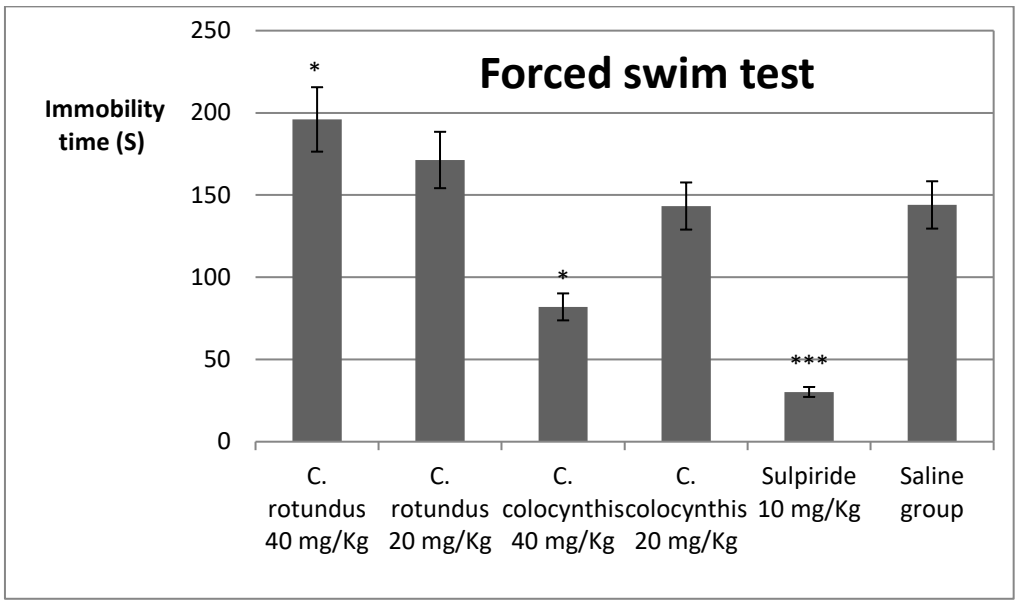


**Figure 1.** Effects of samples on immobility time in the forced swimming test (FST). For statistical significance, *p < 0.05 and ***p < 0.001 when compared to the saline group.

3.2.2. Tail suspension test.

In the tail suspension test, significant differences ($p < 0.001$) were observed between all the tested samples and the saline control group, which exhibited an immobility time of 189.57±9 seconds. The *C. colocynthis* fruit resin group demonstrated superiority over all other samples. Notably, the lower dose of 20 mg/kg exhibited a shorter estimated immobilization time of 8.33 ± 3.51 seconds compared to the higher dose of 40 mg/kg, which had an estimated immobilization time of 57.33± 6.5 seconds. Similar trends were observed with *C. rotundus* tuber resin, where the lower dose outperformed the higher dose. Specifically, at a concentration of 20 mg/kg, the immobility time was 60±6.8 seconds, while the higher concentration of 40 mg/kg resulted in an immobility time of 62.33±5.5 seconds. Interestingly, both doses of *C. rotundus* tuber resin surpassed the group treated with sulpiride at a concentration of 10 mg/kg, which exhibited an immobilization time estimated at 98.74±4.43 seconds (Figure 2).

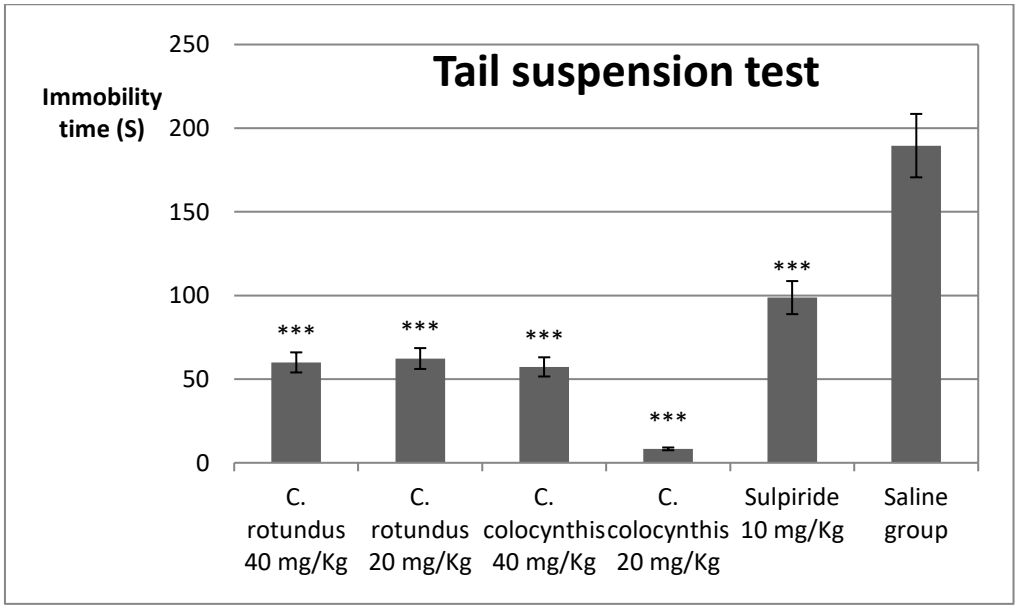


**Figure 2.** Effects of samples on immobility time in the tail suspension test (TST). For statistical significance, ***p < 0.001 when compared to the saline group.

*3.3. In vitro BSA denaturation.*

In relation to the denaturation of BSA, the resin extracted from *C. rotundus* tubers demonstrated an $IC_{50}$ value of 0.543 ± 0.011, showcasing a notable advantage over the denaturation activity observed with *C. colocynthis* Fruit Resin, which registered an approximate $IC_{50}$ value of 0.786 ± 0.026 mg/mL. Noting the superiority of diclofenac sodium, the $IC_{50}$ is estimated at 0.459± 0.018 mg/mL (Figure 3).

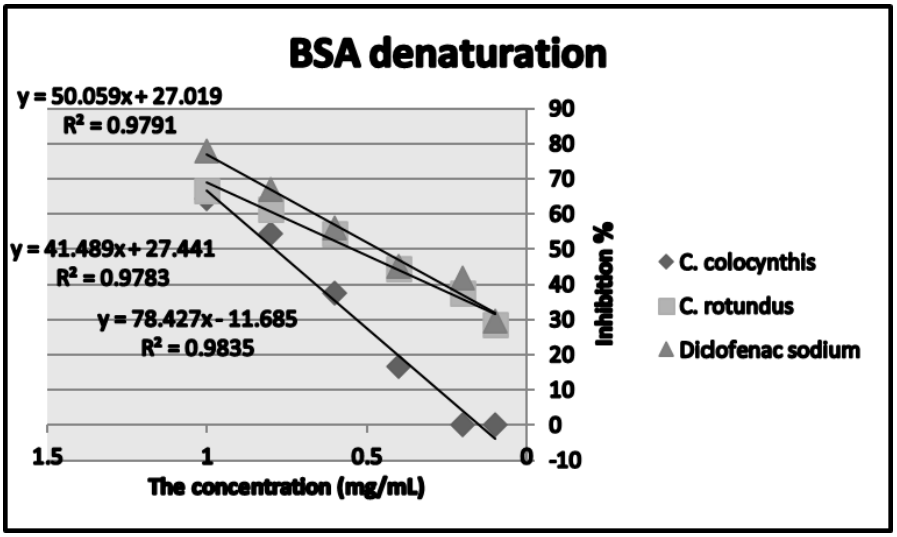


**Figure 3.** Inhibition of Denaturation of BSA activity as a function of concentration.

*3.4. Antimicrobial activity assessment.*

The results illustrate that resins derived from *C. rotundus* and *C. colocynthis* exhibit antimicrobial activity against a range of microorganisms (Table 1). However, their efficacy appears to be comparatively lower when contrasted with the standard antibiotics utilized in this investigation. Gentamicin and Amphotericin B, the two standard antibiotics, consistently demonstrated superior efficacy against all tested microorganisms, as indicated by larger zones of inhibition compared to *C. rotundus* and *C. colocynthis* resins.

**Table 1.**Antimicrobial activity of *C. rotundus* tuber resin and *C. colocynthis* fruit resin.

| **Zone of inhibition in mm (% inhibition)** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Concentration (mg/ml)** | ***C. rotundus*** | | | ***C. colocynthis*** | | | **Gentamicin** (10 µg/disc) | **Amphotericin B** (25 µg/disc) |
| | 0.5 | 1 | 2 | 0.5 | 1 | 2 | | |
| *E. coli* | 7 (6.25) | 7 (6.25) | 9 (18.75) | 7 (6.25) | 8 (12.5) | 10 (25) | 22 | Nd |
| *Ps. aeruginosa* | 8 (20) | 9 (30) | 11 (50) | 8 (20) | 8 (20) | 9 (30) | 16 | Nd |
| *K. pneumoniae* | 6 | 6 | 6 | 6 | 6 | 6 | 29 | Nd |
| *S. aureus* | 6 | 6 | 6 | 6 | 6 | 6 | 30 | Nd |
| *C. albicans* | 14 (50) | 17 (68.75) | 17 (68.75) | 9 (18.75) | 11 (31.25) | 12 (37.5) | Nd | 22 |

The table shows the diameter of the inhibition zone in millimeters. The percentage of inhibition is calculated in comparison to standard antibiotics Gentamicin and Amphotericin. "Nd" means not determined, and the disk diameter is 6 mm (no inhibition).

Both *C. rotundus* and *C. colocynthis* displayed antimicrobial activity against only three of the tested microorganisms (*E. coli*, *Ps. aeruginosa*, and *C. albicans*) across all concentrations. The diameter of the inhibition zone generally increased with higher resin

concentrations for both plant species, suggesting that higher resin concentrations are more effective in inhibiting microbial growth.

Significantly, *C. albicans* exhibited heightened susceptibility to *C. rotundus* resin compared to other microorganisms. Specifically, *C. rotundus* resin achieved the highest inhibition rate (68.75%) against *C. albicans* at a concentration of 2 mg/mL.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, and the conclusions that can be drawn.

*3.5. Discussion.*

The observed differences in behavioral outcomes can be attributed to several factors. Firstly, the distinct responses of the two animal models to specific anti-depressants suggest potential variations in the pharmacological mechanisms underlying their performance in these tests [23]. Secondly, there exists a variance in the sensitivity spectrum to drugs between the two models. Bogdanova et al. [24] and Zheng et al. [25] have documented that swimming behavior in the Forced Swim Test is influenced by serotonergic neurotransmission, whereas climbing behavior is governed by norepinephrinergic neurotransmission. Drugs targeting norepinephrine and those with mixed serotonin reuptake inhibition exhibit greater efficacy in the Tail Suspension Test [26].

Furthermore, administration of 40 mg/kg of *C. rotundus* tuber resin results in a notable reduction in immobility ($p < 0.05$) in the FST, possibly indicating a sedative effect on serotonergic neurotransmission. Hence, it can be inferred that *C. rotundus* resin lacks antidepressant-like actions targeting serotonergic neurotransmission. The observed increase in immobility time may stem from non-specific impacts on the rats' general activity and motor coordination. However, the absence of antidepressant-like effects offers insights into the potential mechanism by which *C. rotundus* tuber resin exerts its sedative actions. It is recognized that many agents enhancing GABAergic transmission induce sedation and drowsiness without displaying anti-depressant effects, both in animals and humans [27]. Nevertheless, further investigations are warranted to corroborate this hypothesis.

Moreover, it has been noted that psychotropic tonics, though clinically ineffective as anti-depressants, demonstrate anti-dyskinetic effects in the TST and FST mediated by stimulants [19,28].

In a study conducted by Ben Ali et al. [14], it was demonstrated that the resin extracted from *C. rotundus* tubers possesses notable antioxidant properties [29]. Furthermore, it was observed that antioxidants exhibit anti-inflammatory effects. Reactive oxygen species (ROS) are well-known to stimulate various intracellular signaling pathways, thereby inducing the expression of proinflammatory cytokines. Additionally, ROS can serve as secondary messengers, triggering the synthesis of additional inflammatory mediators [30]. Plant-derived compounds such as gallic acid, rutin, vanillic acid, quercetin, and kaempferol have been identified as capable of intercepting specific signal transduction events essential for the activation of these inflammatory mediators, consequently exerting anti-inflammatory effects [31,32].

The antibacterial and antifungal properties of the resin type seem to be closely associated with its total phenolic content. This suggests that phenolic compounds, known for

their antioxidant properties, likely play a significant role in the observed antibacterial activity [33].

## 4. Conclusions

Overall, these findings underscore the pharmacological potential of *C. rotundus* tuber resin and *C. colocynthis* fruit resin as candidates for further investigation in the development of novel anti-depressant and anti-inflammatory agents and their potential applications in combating microbial infections. However, comprehensive studies encompassing pharmacokinetic, pharmacodynamic, and safety profiles are imperative to validate their therapeutic utility and clinical translation.

## Author Contributions

All authors have read and agreed to the published version of the manuscript.

## Institutional Review Board Statement

Not applicable

## Informed Consent Statement

Not applicable

## Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

## Funding

This research received no external funding.

## Acknowledgments

The authors express their gratitude to all participants for their indispensable contributions to this study. The Algerian Ministry of Higher Education and the General Directorate of Scientific Research and Technological Development provided support for this research. This investigation is part of the PRFU project with reference number D01N01UN390120220003.

## Conflicts of Interest

The authors declare no conflict of interest.

## Reference

1. Watson, R.; Harvey, K.; McCabe, C.; Reynolds, S. Understanding anhedonia: a qualitative study exploring loss of interest and pleasure in adolescent depression. *Eur. Child Adolesc. Psychiatry* **2020**, *29*, 489-499,https://doi.org/10.1007/s00787-019-01364-y.
2. Chiang, Y.-C.; Liou, T.-H.; Lee, H.-C.; Escorpizo, R. Using WHODAS 2.0 to Assess Functional Impairment in People with Depression: Should Employment Receive More Attention?.*Int. J. Environ. Res. Public Health* **2021**,*18*,4552, https://doi.org/10.3390/ijerph18094552.

3. Cleare, A.; Pariante, C.M.; Young, A.H.; Anderson, I.M.; Christmas, D.; Cowen, P.J.; Dickens, C.; Ferrier, I.N.; Geddes, J.; Gilbody, S.; Haddad, P.M.; Katona, C.; Lewis, G.; Malizia, A.; McAllister-Williams, R.H.; Ramchandani, P.; Scott, J.; Taylor, D.; Uher, R. Evidence-based guidelines for treating depressive disorders with antidepressants: A revision of the 2008 British Association for Psychopharmacology guidelines. *J. Psychopharmacol.* **2015**, *29*, 459-525, https://doi.org/10.1177/0269881115581093.
4. Martins, J.;Brijesh, S. Phytochemistry and pharmacology of anti-depressant medicinal plants: A review. *Biomed. Pharmacother.* **2018**,*104*,343-365, https://doi.org/10.1016/j.biopha.2018.05.044.
5. Feng, D.-d.; Tang, T.; Lin, X.-p.; Yang, Z.-y.; Yang, S.; Xia, Z.-a.; Wang, Y.; Zheng, P.; Wang, Y.; Zhang, C.-h. Nine traditional Chinese herbal formulas for the treatment of depression: an ethnopharmacology, phytochemistry, and pharmacology review. *Neuropsychiatr. Dis. Treat.* **2016**, *12*, 2387-2402,https://doi.org/10.2147/NDT.S114560.
6. Kamala, A.; Middha, S.K.; Karigar, C.S. Plants in traditional medicine with special reference to *Cyperus rotundus* L.: a review. *3 Biotech* **2018**, *8*, 309,https://doi.org/10.1007/s13205-018-1328-6.
7. Kapoor, M.; Kaur, N.; Sharma, C.; Kaur, G.; Kaur, R.; Batra, K.; Rani, J. Citrullus colocynthis an Important Plant in Indian Traditional System of Medicine. *Pharmacogn. Rev.* **2020**, *14*, 22-27, https://doi.org/10.5530/phrev.2020.14.4.
8. Ben Ali, A.; Chouikh, A. Bioactive Potential of Algerian Citrullus colocynthis Resin: Antioxidant & Anti-inflammatory Effects. *Notulae Scientia Biologicae*. **2024**, *16(3)*, 132-139, https://doi.org/0.15835/nsb16311782.
9. Gacem, M.A.; Ould El Hadj-Khelil, A.; Boudjemaa, B.; Gacem, H. Phytochemistry, Toxicity and Pharmacology of *Pistacia lentiscus*, *Artemisia herba-alba* and *Citrullus colocynthis*. In *Sustainable Agriculture Reviews 39*, Lichtfouse, E., Ed.; Springer International Publishing: Cham, **2020**; Volume 39, pp. 57-93,https://doi.org/10.1007/978-3-030-38881-2_3.
10. Siroua, K.; Belamine, H.; Kadiri, F.; Ait Haj Said, A.; Blaghen, M.; El Kouali, M.; Kenz, A. Antioxidant and antibacterial activities evaluation of the hydroethanolic extract of *Cyperus rotundus* L. and its fractions. *Vegetos* **2022**, *35*, 633-641,https://doi.org/10.1007/s42535-022-00356-9.
11. Ferreira, M.F.; Castanheira, L.; Sebastiao, A.M.; Telles-Correia, D. Depression Assessment in Clinical Trials and Pre-clinical Tests: A Critical Review. *Curr. Top. Med. Chem.* **2018**, *18*, 1677-1703,https://doi.org/10.2174/1568026618666181115095920.
12. Stukalin, Y.;Lan, A.;Einat, H. Revisiting the validity of the mouse tail suspension test: Systematic review and meta-analysis of the effects of prototypic antidepressants. *Neurosci. Biobehav. Rev.* **2020**,*112*,39-47, https://doi.org/10.1016/j.neubiorev.2020.01.034.
13. Yan, H.-C.; Cao, X.; Das, M.; Zhu, X.-H.; Gao, T.-M. Behavioral animal models of depression. *Neurosci. Bull.* **2010**, *26*, 327-337, https://doi.org/10.1007/s12264-010-0323-7.
14. Ben Ali, A.; Chouikh, A.; Haddad, L. *Cyperus rotundus* tubers resin from Algeria: a promising source of natural antioxidants, anti-inflammatory, and photoprotective compounds. *Ovidius University Annals of Chemistry* **2023**, *34*, 132-139, https://doi.org/10.2478/auoc-2023-0017.
15. Lorke, D. A new approach to practical acute toxicity testing. *Arch. Toxicol.* **1983**,*54*,275-287, https://doi.org/10.1007/BF01234480.
16. Ben Ali, A; Chouikh, A.; Haddad, L. *Citrullus colocynthis* fruit resin and *Cyperus rotundus* tuber resin: Assessing their analgesic effects with hot plate and writhing tests. *Acta periodica technologica*. **2024**,*55(1)*, 1-12, https://doi.org/10.2298/APT2455001B.
17. Chouikh, A.; Ben Ali, A.; Brahim Bousbia, A. Exploring Therapeutic Potential of Malcolmia Aegyptiaca Spr. and Matthiola Livida DC. Extracts in Rat Models Using Hot-Plate, Writhing and Carrageenan-Induced Paw Edema Tests. *Acta Medica Bulgarica*. **2024**,*51(Suppl 2)*,102-109, https://doi.org/10.2478/amb-2024-0060.
18. Porsolt, R.D.;Le Pichon, M.;Jalfre, M. Depression: a new animal model sensitive to antidepressant treatments. *Nature***1977**,*266*,730-732, https://doi.org/10.1038/266730a0.
19. Cryan, J.F.;Mombereau, C.;Vassout, A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neurosci. Biobehav. Rev.* **2005**,*29*,571-625, https://doi.org/10.1016/j.neubiorev.2005.03.009.
20. Agbaje, E.O.;Ishola, I.O.;Oniyire, J.A. Antidepressant, anxiolytic, and anticataleptic effects of aqueous leaf extract of *Antiaris toxicaria* Lesch.(Moraceae) in mice: possible mechanisms of actions. *J. Basic Clin. Physiol. Pharmacol.***2014**,*25*,429-438, https://doi.org/10.1515/jbcpp-2013-0054.

21. Chouikh, A.; Ben Ali, A.; Brahim Bousbia, A.; Khezzani, K.; Bekkouche, S. Comparative analysis of phytochemical composition and biological activities of sprouted and unsprouted Chenopodium quinoa willd. seeds: insights into nutritional value and functional properties Acta Universitatis Cibiniensis Series E: Food Technology. **2024**,*28(1)*,55-64, https://doi.org/10.2478/aucft-2024-0005.
22. Hudzicki, J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol. *Am. Soc. Microbiol*.**2009**,15,1-23.
23. Bai, F.; Li, X.; Clay, M.; Lindstrom, T.; Skolnick, P. Intra- and interstrain differences in models of "behavioral despair". *Pharmacol. Biochem. Behav.* **2001**, *70*, 187-192,https://doi.org/10.1016/S0091-3057(01)00599-8.
24. Bogdanova, O.V.; Kanekar, S.; D'Anci, K.E.; Renshaw, P.F. Factors influencing behavior in the forced swim test. *Physiol. Behav.* **2013**, *118*, 227-239, https://doi.org/10.1016/j.physbeh.2013.05.012.
25. Zheng, J.-Y.; Li, X.-X.; Liu, X.; Zhang, C.-C.; Sun, Y.-X.; Ma, Y.-N.; Wang, H.-L.; Su, Y.-A.; Si, T.-M.; Li, J.-T. Fluoxetine reverses early-life stress-induced depressive-like behaviors and region-specific alterations of monoamine transporters in female mice. *Pharmacol. Biochem. Behav.* **2024**, *237*, 173722, https://doi.org/10.1016/j.pbb.2024.173722.
26. Gao, N.; Tiliwaerde, M.; Zheng, W.; Xiong, J.; Li, X.; Jin, Z. Neuropharmacological and antidepressant-like effects of ZY-1408: A novel serotonin/norepinephrine reuptake inhibitor and serotonin receptor 2C antagonist. *Neuropharmacology* **2021**, *182*, 108376,https://doi.org/10.1016/j.neuropharm.2020.108376.
27. Kaur, S.; Singh, S.; Arora, A.; Ram, P.; Kumar, S.; Kumar, P.; Abed, S.N. Pharmacology of GABA and Its Receptors. In *Frontiers in Pharmacology of Neurotransmitters*, Kumar, P., Deb, P.K., Eds.; Springer Singapore: Singapore, **2020**; pp. 241-292,https://doi.org/10.1007/978-981-15-3556-7_8.
28. Sherman, A.D.; Sacquitne, J.L.; Petty, F. Specificity of the learned helplessness model of depression. *Pharmacol. Biochem. Behav.* **1982**, *16*, 449-454, https://doi.org/10.1016/0091-3057(82)90451-8.
29. Thring, T.S.A.; Hili, P.; Naughton, D.P. Antioxidant and potential anti-inflammatory activity of extracts and formulations of white tea, rose, and witch hazel on primary human dermal fibroblast cells. *J. Inflamm.* **2011**, *8*, 27, https://doi.org/10.1186/1476-9255-8-27.
30. Winrow, V.R.; Winyard, P.G.; Morris, C.J.; Blake, D.R. Free radicals in inflammation: second messengers and mediators of tissue destruction. *Br. Med. Bull.* **1993**, *49*, 506-522, https://doi.org/10.1093/oxfordjournals.bmb.a072627.
31. Sivamani, S.; Joseph, B.; Kar, B. Anti-inflammatory activity of *Withania somnifera* leaf extract in stainless steel implant induced inflammation in adult zebrafish. *J. Genet. Eng. Biotechnol.* **2014**, *12*, 1-6, https://doi.org/10.1016/j.jgeb.2014.01.002.
32. Chouikh, A.; Ben Ali, A.; Brahim Bousbia, A.; Bekkouche, A.; Seghaier, S. Phytochemical analysis and biological activities of Matthiola livida DC. extracts from Oued-Souf region: Insights into antioxidant and anti-inflammatory potential. Acta periodica technologica. **2024**, *55(1)*,107-124, https://doi.org/10.2298/APT2455107C.
33. Selles, C.; Dib, M.E.A.; Allali, H.; Tabti, B. Evaluation of antimicrobial and antioxidant activities of solvent extracts of Anacyclus pyrethrum L., from Algeria. *Mediterr. J. Chem.* **2012**, *2*, 408-415, https://doi.org/10.13171/mjc.2.2.2012.01.11.22.


## Publisher's Note & Disclaimer