

**BIOLOGICAL POTENTIAL OF *BRACHIARIA RAMOSA* (LINN.) STAFF:
CYTOTOXIC, ANTIFUNGAL, ANALGESIC PROPERTIES OF
METHANOLIC AND ETHANOLIC EXTRACTS**

**SYED AWAIS SHAH*, FAZAL MALIK SARIM, MUHAMMAD ADIL, SAYED QASIM SHAH,
SIDRA KHAN DURRANI AND ADIL MUHAMMAD**

*Department of Chemical and Life Sciences, Qurtuba University of Science and Information
Technology, Hayatabad, Peshawar, Khyber Pakhtunkhwa, Pakistan*

Keywords: Brachiaria ramosa, Cytotoxic, Antifungal, Analgesic properties

Abstract

The work was carried out to explore the cytotoxic, antifungal and analgesic activities of *Brachiaria ramosa*. The extracts were applied against brine shrimp nouppli, fungal strains and mices. The cytotoxic potential showed that LD₅₀ value of various concentrations target to die 50% of the larvae. The antifungal activity of *B. ramosa* revealed that the methanol extract represent the highest zone of inhibition (24.55 ± 0.66 mg/ml) against *Acremonium alternatum* followed by ethanol extracts against *Rhizophus nigricans* (21.70 ± 1.34). The analgesic activity of the *B. ramosa* showed results after 90 min (1.95 ± 0.04) at 300 mg/kg. Both the extracts of *B. ramosa* showed significant cytotoxic, antifungal and analgesic activities.

Cytotoxicity is the ability of being selected and non-selected products to living organism, divides an essential factor in the biological and pharmaceutical investigations (Castro-Díaz *et al.* 2025). The investigation of the cytotoxic activity serves as essential preliminary display tool in identification of drugs models basically in the determination of great verities of compounds with potential and curing applications (Alonso-Castro *et al.* 2012). Cytotoxic substances play a key role through their assorted and often connected mechanisms including the creating oxidative stress for example reactive oxygen species (Ding *et al.* 2020). The ability to trigger the programmed cell death selected the infected cells while wasting the cells of host remains the ultimate aims in cytotoxic agents' development underscoring the need of comprehensive and systematic bioactivity profiling (Muhammad *et al.* 2024). Pathogens are the microorganisms form a parasitic association with other organism's e.g. harmful fungi causes different type of infections in immunosuppressed patients where fungi causes many human diseases (Okeke and Ezeabara 2018). Mostly human disease are causes due to the attack of fungi and other microorganism most therapists treat various fungal infections due to this it may cause multiple problems and also prepare resistant strains which is not important as other Alternately, with the use of antibiotics in greater numbers and the risk capacity of the antibiotics some clinical substances are used to determine the novel drugs to solve the problems ((Nikolova *et al.* 2022). The drugs that selectively reduce pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness are called analgesics (Venkatachalam *et al.* 2018). Most synthetic drugs have represented different health issues or side effect. Therefore, pain relievers have low side effects and are essential (Salwe *et al.* 2018). Plants extracts are known as alternative substances for removing and have less side effects (Zihad_ *et al.* 2018).

Brachiaria ramosa (Linn.) Staff was collected from Dandaw, Takht Bhai, Mardan, Pakistan in June 2023. After washing, they were shade dried and grind into powder. The powder was successively extracted with methanol and ethanol. The extraction of plants materials was first filter through filter paper and then by filter paper 41. By rotatory evaporator under the absence of air

*Author for correspondence: <a.shah3277@gmail.com>. ¹Department of Life and Chemical, Sciences Qurtuba University of Science and Information Technology Hayatabad, Peshawar, Khyber Pakhtunkhwa, Pakistan.

solvent were completely vaporized to obtain the crude extract. The filtrate acquired were kept in a refrigerator (Chuoudhry *et al.* 2011).

The required tray also known as hatching tray half filled with sodium chloride solution. The tray were partitioned which were separated into two different perforated parts. The larger half part was kept open and smaller part were sprinkled with 25 mg of concentrated salt solution (shrimp) egg and protected through black paper. Open portion of the tray were illuminated by a lamp that were placed above it. The nouppli swarm activity was seen moving through the perforated divider toward the lit portion after hatching (Atta-ur-Rahman *et al.* 2005).

Antifungal activity was performed using methanol and ethanol extraction of *B. ramosa* following the methodology of Mahesh and Satish (2008). Test fungal strains i.e., *Acremonium alternatum*, *Rhizopus nigricans*, *Trichoderma viride* and *Aspergillus niger* were obtained from Abasin University Lab and Biotechnology Center of Agriculture University.

Analgesic activity on albino mice was carried out on hot plat following the method of Bianchi *et al.* (1954). The albino mice were divided into five groups each group consisting of five mice. Group one was taken as a control and administered in normal saline solution (10 ml/ kg). Group 2 was treated with standard drugs diclofenac sodium (25 mg /kg) and group 3, 4 and 5 received oral doses of methanol and ethanol extracts at 100, 200, and 300 mg per Kg of body weight respectively, The mice were placed on hot plat retained at $55 \pm 0.5^{\circ}\text{C}$ to record the base line reaction time for the every group. The mice responses to the heat were determined on behavior such as jumping. After the treatment the reaction time were calculated again 30, 60 and 90 min.

$$\text{Elongation (\%)} = \frac{(\text{Latency (test)} - \text{Latency (control)})}{\text{Latency (test)}} \times 100$$

The methanol extract of different concentration 50, 100, and 150 $\mu\text{g/ml}$ were analyzed and represented (30), (46.6) and (63.3 %) 150 $\mu\text{g/ml}$ was dose-dependent because it represents the highest death rate in brine shrimp napullii. The LD_{50} value reported for the methanol extract of *Brachiaria ramosa* was 110.18 $\mu\text{g/ml}$ while the ethanol extract of the plant showed (40) (53) and (73%) cytotoxic potential to kill the brine shrimp napulli. 150 $\mu\text{g/m}$ was dose-dependent because it represents the highest death rate in brine shrimp napullii Table 1. The LD_{50} value reported which is the concentration target to die 50% of the larvae for ethanol extract the value was determined 92.5 g/ml. This value showed a moderate level of cytotoxicity representing that higher concentration increases the level of mortality similar findings are in with conformity with ME *et al.* (2025) studied the cytotoxic potential of various fractions of leaves of *Ziziphus mauritiana* (Lam) the study revealed the reduction in the mitotic cell line.

Table 1. Cytotoxic potential of *Brachiaria ramosa* extracts against larvae.

Extracts	Conc. ($\mu\text{g/ml}$)	No. of larvae	Dead larvae	Survived larvae	Mortality rate (%)	LD_{50} ($\mu\text{g/ml}$)
Methanol extract	50	30	9	21	30	110.18
	100	30	14	16	46.6	
	150	30	19	11	63.3	
Ethanol extract	50	30	12	18	40	92.5
	100	30	16	14	53	
	150	30	22	8	73	

The antifungal activity of methanol and ethanol extracts of various concentrations (100 mg/ml, 200 mg/ml, 300 mg/ml) applied against the fungal strains *Acremonium alternatum*, *Rhizophus nigricans*, *Trichoderma viride* and *Aspergillus niger*. The methanol extract represent the highest zone of inhibition (24.55 ± 0.66 mg/ml) against *Acremonium alternatum*, followed by

Rhizopus nigricans and *Trichoderma viride* (23.92 ± 1.43 mg/ml), (23.03 ± 0.84) and (22.10 ± 1.25) and the lowest zone of inhibition (5.37 ± 1.03 mg/ml) was measured against *Aspergillus niger* at concentrations of (100 mg/ml) represents. The ethanol extracts of *Brachiaria ramosa* represent the highest zone of inhibition (21.70 ± 1.34) was measured against the *Rhizopus nigricans* followed by *Aspergillus niger*, *Trichoderma viride*, and *Acremonium alternatum* (21.10 ± 1.32), (20.17 ± 0.71) and (19.54 ± 0.96) the lowest zone of inhibition (5.45 ± 0.64) shown by *Acremonium alternatum* at minimum concentrations at 100 mg/ml in Table 2. These findings agreed with Al-Askar *et al.* (2023) who evaluated *E. campestre* for antifungal activity. The result suggested the highest zone of inhibition against *R. solani* fungus and less effective fungal growth inhibition about 78.52 %.

Table 2. Effects of *Brachiaria ramosa* extracts against fungi.

Extracts	Fungi	Inhibition zone at different concentrations			+ve Control
		100 mg/ml	200 mg/ml	300 mg/ml	
Methanol extract	<i>Acremonium alternatum</i>	7.31 ± 1.16	16.30 ± 1.68	24.55 ± 0.66	30
	<i>Rhizopus nigricans</i>	7.41 ± 0.71	15.88 ± 0.94	23.92 ± 1.43	30
	<i>Trichoderma viride</i>	6.78 ± 1.70	16.32 ± 0.87	22.10 ± 1.25	30
	<i>Aspergillus niger</i>	5.37 ± 1.03	15.40 ± 0.79	23.03 ± 0.84	30
Ethanol extract	<i>Acremonium alternatum</i>	5.45 ± 0.64	13.24 ± 0.79	19.54 ± 0.96	30
	<i>Rhizopus nigricans</i>	6.07 ± 1.74	15.13 ± 1.18	21.70 ± 1.34	29
	<i>Trichoderma viride</i>	6.26 ± 1.34	15.14 ± 1.33	20.17 ± 0.71	29
	<i>Aspergillus niger</i>	7.50 ± 1.64	15.03 ± 1.15	21.10 ± 1.32	30

The methanol extract of *Brachiaria ramosa* showed increased the latency time after 60 min (6.60 ± 1.25) at 200 mg/kg, (5.76 ± 0.58) at 300 mg/kg and after 90 min all doses showed (7.92 ± 3.45) at 100 mg/kg, (4.24 ± 1.25) at 200 mg/kg and (9.10 ± 3.99) at 300 mg/kg as compared to the standard drugs present in the Table 3. The maximum percent increase 33, 43 and 47% in the latency time while methanol extract was shown after 90 min of administration as correlated to the standard drug of diclofenac which exhibited the percentage increase in 59%. Whereas, the ethanol extract showed a significant increase in the latency time after 60 min (5.02 ± 1.88) at 200 mg/kg and (5.44 ± 3.00) at 300 mg/kg and after 90 min all the doses (6.74 ± 3.02) at 100 mg/kg, (8.50 ± 1.63) at 200 mg/kg and (9.78 ± 5.10) at 300 mg/kg as compared to the standard drugs present in the Table 3. The maximum percent increase in the 12, 25, and 49% latency time of ethanol extract was showed after 90 min of administration as correlated to the standard drugs of diclofenac which exhibited the percentage increase in 49%. These findings conformity with Ferdous *et al.* (2018) and Ferdous *et al.* (2020) who evaluated *A. calcarata* and confirm different phytochemicals in the plant could be responsible for analgesic activity and dose dependent.

Table 3. Analgesic effects of *Brachiaria ramosa*.

Treatments	Doses	30 min	60 min	90 min	Inhibition (%)
Normal saline	10	3.80 ± 0.89	5.18 ± 2.90	5.00 ± 2.98	04.66
Diclofenic sodium	25	5.40 ± 0.54	6.62 ± 0.44	10.32 ± 0.37	59
Methanol extract	100	5.72 ± 3.37	5.02 ± 1.20	7.92 ± 3.45	33
	200	6.46 ± 1.16	6.60 ± 1.25	4.24 ± 1.25	43
	300	5.72 ± 1.13	5.76 ± 0.58	9.10 ± 3.99	47
Ethanol extract	100	3.92 ± 2.79	5.02 ± 1.88	6.74 ± 3.02	12
	200	4.04 ± 1.01	5.02 ± 1.88	8.50 ± 1.63	25
	300	5.72 ± 2.93	5.44 ± 3.00	9.78 ± 5.10	49

The current study conclude that methanol and ethanol extracts of *Brachiaria ramosa* showed good result in cytotoxic potentials, antifungal activity and analgesic activity. In future it is essential to formulate this plant help in ant cancerous, antifungal and pain reliever drugs.

Acknowledgments

The authors would like to represent heartfelt appreciation to Department of Chemical and life Sciences, Qurtuba University of Science and Information Technology, Peshawar, Khyber Pukhtoonkhwa, Pakistan.

References

- Alonso-Castro AJ, Maldonado-Miranda JJ, Zarate-Martinez A, del Rosario Jacobo-Salcedo M, Fernández-Galicia C, Figueroa-Zuñiga LA and Carranza-Alvarez C 2012. Medicinal plants used in the *Huasteca Potosina* México. *J. Ethnopharma.* **143**(1): 292-298.
- Al-Askar AA, Bashir S, Mohammad AE, Sharaf OA, Nabil R, Su Y and Behiry SI 2023. Antimicrobial efficacy and HPLC analysis of polyphenolic compounds in a whole plant extract of *Eryngium campestre*. *Separations* **10**(6): 362.
- Atta-ur-Rahman NH, Fadimatou MT, Yasin A, Fatim N and Chaudhry MI 2005. Bioactive constituents from *Boswellia papyrifera*. *J. Nat. Prod.* **68**(2): 189-193.
- Bianchi C and Frances Chini J 1954. Experimental observations on Haffner's method for testing analgesic drugs. *Brit. J. Pharm. Chem.* **9**(3): 280.
- Castro-Díaz R, Silva-Beltrán N P, Gámez-Meza N and Calderón K 2025. The antimicrobial effects of coffee and by-products and their potential applications in healthcare and agricultural sectors: a state-of-art review. *Microorganism* **13**(2): 215.
- Ding D, Mutrie N, Bauman A, Pratt M, Hallal PR and Powell KE 2020. Physical activity guidelines 2020: comprehensive and inclusive recommendations to activate populations. *The Lancet* **396**(10265): 1780-1782.
- Choudhury N and Sekhon BS 2011. An overview of advances in the standardization of herbal drugs. *J. Pharma. Edu. Res.* **2**(2): 55.
- Ferdous M, Basher MA, Khan I, Ahmed F, Sobuz MSI and Daula ASU 2018. Evaluation of phytochemicals antioxidant and antibacterial potential of *Alpinia calcarata*. *J. Med. Plant. Stud.* **6**(2):152-158.
- Ferdous Mahmuda FM, Shahid Ud Daula, Shahnaz N, Farjana Y and Mohammad AB 2020. Analgesic, anxiolytic and sedative-like activities of leaves of *Alpinia calcarata* Roscoe in mice. *J. Med. Plants Res.* **14**(4): 155-163.
- ME S, El-Sherbiny GM, Sharaf MH, Kalaba MH and Shaban AS 2025. Phytochemical analysis, antimicrobial, antioxidant, and cytotoxicity activities of *Schinus molle* (L.) extracts. *Biom. Conver. Bioref.* **15**(3): 3753-3770.
- Muhammad M, Waheed A, Wahab A, Majeed M, Nazim M, Liu YH and Li WJ 2024. Soil salinity and drought tolerance: An evaluation of plant growth, productivity, microbial diversity, and amelioration strategies. *Plant Stress* **11**: 100319.
- Mahesh B and Satish S 2008. Antimicrobial activity of some important medicinal plant against Plant and human pathogens. *World. J. Agric. Sci.* **4**(5): 839-843.
- Nikolova M, Todorova Y, Emilova R, Trifonova I, Gladnishka T, Petrova-Yancheva, N and Christova I 2022. Induction of humoral and cellular immune responses to COVID-19 mRNA and vector vaccines: A prospective cohort study in Bulgarian healthcare workers. *J. Med. Virol.* **94**(5): 2008-2018.
- Okeke IC and Ezeabara CA 2018. Phytochemical screening and *in vitro* antimicrobial activity of various parts of *Cleome ciliata* Schum. & Thonn. *Bioscience Horizons: Int. J. Stud. Res.* **11**: hzy018.
- Salwe S, Padwal V, Nagar V, Patil P and Patel V 2019. T cell functionality in HIV-1, HIV-2 and dually infected individuals: correlates of disease progression and immune restoration. *Clin. Exper. Immun.* **198**(2): 233-250.

- Venkatachalam K, Vinayagam R, Arokia Vijaya Anand M, Isa NM and Ponnaiyan R 2020. Biochemical and molecular aspects of 1, 2-dimethylhydrazine (DMH)-induced colon carcinogenesis: a review. *Toxi. Res.* **9**(1): 2-18.
- Venkatachalam D, Thavamani BS and Krishniah M 2018. Evaluation of analgesic activity of ethanolic and aqueous extracts of leaf of *Plumeria rubra* in albino rat. *Pharma. Biol. Eval.* **5**(2): 52-58.
- Zihad SNK, Bhowmick N, Uddin SJ, Sifat N, Rahman MS, Rouf R and Sarker S D 2018. Analgesic activity, chemical profiling and computational study on *Chrysopogon aciculatus*. *Front. In. Pharma.* **9**: 1164.

(Manuscript received on 30 April, 2026; revised on 11 September, 2026)