In *Vitro* Efficacy Of Bioactive Extracts Of *BrachiariaLata* (Schumach.) C.E.Hubb. Against Athogenic Bacteria And Fungi

A. Gauttam[1]*,H. Singh[2]

Abstract

This study was carried out to evaluate the antibacterial and antifungal potentials ofBrachiarialata (Schumach.) C.E.Hubb (B. lata). In the current study, the methanol and chloroform extracts of leaves and stem B. latawereevaluatedfor possible antimicrobial activity against strains of bacteria and fungus. Theantimicrobial potential was evaluated in the leaves and stem extracts using the agar well disc diffusion method. The antibacterialand antifungal potential of extracts (20 mg/ml) were tested against five bacterial strains such asBacillus subtilis, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus, and Streptomyces and four fungal strains such asPenicillium chrysogenum, Aspergillus niger, Fusarium oxysporum, and Candida albicans. Zone of inhibition of extracts was compared with that of standards ciprofloxacin (10 mg/discs) for antibacterial activity and ketoconazole (10 µl)for antifungal activity. The results showed that the remarkable inhibition of thebacterial growth was shown against the tested organisms while not having significant antifungal activity.

Keywords: Brachiarialata (Schumach.) C.E.Hubb., Antibacterial, Antifungal, Grass
DOI Number: 10.48047/nq.2022.20.19.NQ99246 **NeuroQuantology2022;20(19): 2895-2899**

Introduction

Brachiarialata (Schumach.) C.E.Hubb. (Family: Poaceae, *Brachiarialata*) is a common yearly grass and distributed in northern tropical Africa, Egypt, Sudan, Eritrea, Arabia and Ethiopia (Airy-Shaw, 1939).*Brachiarialata*(*B. lata*) is accepted name and having many synonyms like *Panicumamplexifolium*, *Panicum exasperatum*Nees, *Panicum hamadense*Mez, *Panicum insculptum*, *Panicum latum,Urochloainsculpta*, and *Urochloalata*(http://www.worldfloraonline.org /taxon/wfo-0000854151). *B.lata*is an annual grass 20 to 60 cm in height and found in upland ecology (Nwilene*et al.,* 2009).*B.lata* have a procumbent growth habit, meaty and firm culms, wide blades, and firmly exserting inflorescences with racemes bearing crowded spikelets in pairs or small clusters. It contains n=24 chromosomes(Basappa*et al.,* 1987).*B.lata* is rare in Rajasthan, India, and found in sandy to rocky habitatsin the desert of Western Rajasthan. It was reported in Ranthambhore Tiger Reserve, Rajasthan, India (Shrivastava and Singh, 2009). Some of the species of Brachiaria genera are reported as medicinal plants, including bark and roots of*Brachiariabrizantha*chopped and poundedused forscabies (Agize*et al.,* 2013). Roots of *Brachiariabrizantha*crushed and taken orally for children and chewing the root by adults for stomach ache, anaphylactic shock and epilepsy (Andarge*et al.,* 2015). Amjad (2015) reported, *Brachiariaeruciformis* Sm. used as antioxidant and antibacterial agent. *Brachiariareptans*have Laxative and diuretic property as reported by Ajaib *et al.*(2014).Freshly crushed stem juice of *Brachiariamutica*used in eye drop to cure the watering of the eyes of cattle (Mitra and Mukherjee, 2009).Leaves of *Brachiariadistichophylla* used to apply tothewound. In vitro activity of *Brachiariadistichophylla* shows antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli* (Adamu *et al.,* 2005). Methanolic extract of Brachiaria *sp.* evaluated for antibacterial activity, and it was active against *Staphylococcus aureus*, *Escherichia coli*,

***Corresponding author:-**A. Gauttam
Address:[1*,2]Department of Agriculture, Vivekananda Global University, Jaipur (India), e-mail: anuradhagoutam123@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

pneumoniae(Doss *et al.,* 2012). Leave of Brachiaria sp. used for methanolic extraction, and further, it was utilized to evaluate antibacterial activity. It was reported active against *Staphylococcus aureus* and *Escherichia coli*. Minimum inhibitory concentration was also reported for methanolic extract, and it was 0.5mg/ml and 1.0mg/ml against *Staphylococcus aureus* and *Escherichia coli*, respectively (Mubarack*et al.,* 2011).To date, there is no report or study conducted for in vitro antimicrobial activity of *B.lata*.The present study focused ontheantimicrobial effects of leaf and stem'smethanol and chloroform extract of *B.lata*.

Material and Methods

Sample Collection and identification

*B.lata*collected from Sawaimadhopur district of Rajasthan, India during the August to October 2017. Healthy plants are collected from fertile land. These collected plant material authenticated by the Department of Botany, the University of Rajasthan.

Drying and Grinding of the Plant

The collected leaf and stems were sliced into tiny pieces by using scissors and knives. They were kept for drying under shade to avoid and protect from surrounding contamination and dust in the environment. Shade drying was done in a room for about two weeks without any exposure to light. After completely drying the plants, grinding was done to obtain powder in uniform size and to enhance the surface area for a better extraction process.

Soxhlet Extraction

20 g of finely ground uniform-size range particle containingpowder of the plant sample is put ina porous bag made from cellulose strong filter then kept in a thimble chamber ofSoxhletappratus. Extraction was accomplished in 200 ml of solvent (methanol and chloroform)fill inthe round bottom flask of Soxhletappratus. The upper part was assembled with acondenserconnect to water inflow and outflow. Thesolvent was burning at a moderate temperature around 40°C, and the solvent evaporates slowly and entered intothethimble chambercontaing sample pouch where iscondensed, andreturned back when thesolvent containing extracts reaches the siphon arm of condenser apparatus and empty into round bottom flask and this process repeat again and again. The process was continuously runfor 24hrs until clear solvent drop observed. Furthermore, forwarding on the next step, the extract was filtered to get dried extract to analyze biological activities. It was stored in an airtight bottle at 4°C.

Antibacterial Activity

Preparation of Extracts Solution

The dried extracts obtained were dissolved to make a solution of concentration in dimethylsulfoxide (DMSO) of 20 mg/ml. For appropriate mixing, the solution was held in a centrifuge for 20 minutes at 10000 rpm. The standard antibiotics ciprofloxacin (10 mg/discs) were employed for assessment of the potential with each fraction.

Test organisms

The microorganisms used in the study were cultivated in the Department of Microbiology, Vivekananda Global University, Jaipur, Rajasthan. The bacteria used was *Bacillus subtilis*(MTCC-9878), *Streptococcus pyogenes*(MTCC-1928), *Escherichia coli*(MTCC-9721), *Staphylococcus aureus*(MTCC-9760),and*Streptomyces*(MTCC-8589).

Antibiotic Susceptibility Testing

Microorganism suspension was prepared as McFarland standard for the antibacterial sensitivity test analysis.The MHA (Muller–Hinton Agar) was used for bacterial media preparation. The culture media were prepared in 500 ml distilled water by dissolving 19 g of MHA in a 1000 ml conical flask. The obtained solution was mixed meticulously and stewed with frequent agitation to melt agar completely, and a clear to somewhatopaline gel mix obtained. Then, autoclave the MHA media for sterilization at 121°C temperature,15 lbs pressure for 15 minutes. After sterilization, sterilized media allow to lukewarm at room temperature in a laminar air flow hood. Now dispense 25 ml of the MHA media into each sterilized petri plate. Petri plate containing MHA medialeave for some time to allow for solidification. After solidification, inoculate the petri plated by spreading the culture microbes suspension on media using cotton swabs and cover the whole media with turn 90° degree rotation without leaving any gap. Make three bores in each petri plate separated from each

other. 30 µl of each fraction is dispensed in the two bores, and antibiotics in the middle bore for control. Store all Petri plates for incubation in biochemical oxygen demand (BOD) incubator at 37°C for 24 hrs (Hassan and Ullah, 2019).

Antifungal Activity

The antifungal activity was performed on Potato Dextrose Agar with the fungus*Penicillium chrysogenum*(MTCC-6891), *Aspergillus niger*(MTCC-9933), *Fusarium oxysporum*(MTCC-9966), and *Candida albicans*(MTCC-7253)cultivated in the Department of Microbiology, Vivekananda Global University, Jaipur, Rajasthan.The well-diffusion was carried out as a standard protocol for the evaluation of antifungal activity of plant extracts. The culture was done asMcFarland standard sterilized media prepared at 121°C for14 minutes in an autoclave. 30 µl of extracts was used for analysis, and 10µl of ketoconazole was used as the standard sample. Store all petri plates for an incubation period of 48 hrs attemperature 28°C(Scorzoni*et al.,* 2007).

Results and discussion

Antibacterial activity of leaf extracts of *B.lata*issummarized in Table-1, Figure-1, and Figure-2. Methanol extracts of *B.lata*leave show the maximum zone of inhibition (3.4± 0.1) against *Bacillus subtilis* and*Streptococcus pyogenes* followed by *Escherichia coli* (ZOI-3.05±0.15) and *Staphylococcus aureus* (ZOI-3± 0.1), which is less as compares to control. Similar results were also reported by Mubarack*et al.*(2011) for methanolic leave of Brachiaria sp. used to evaluate antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. They also reported minimum inhibitory concentration for methanolic extract, and it was 0.5mg/ml and 1.0mg/ml against *Staphylococcus aureus* and *Escherichia coli*, respectively (Mubarack*et al.,* 2011).Chloroform extracts of*B.lata*leave exhibits maximum zone of inhibition (ZOI-3.2±0.1) against followed by *Streptococcus pyogenes* followed by *Escherichia coli* (ZOI-2.65±0.55) and *Bacillus subtilis* (ZOI-2.35±0.05) which is not as much of control.Doss *et al.* (2012) reported a methanolic extract of Brachiaria *sp.*shows antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus sp*. and *Klebsiella pneumoniae*.

Antibacterial activity of stem extracts of *B.lata*is represented in Table-2, Figure-3, and Figure-4. Antibacterial activity of methanol extracts of *B.lata* stem displays maximum against *Streptococcus pyogenes* (ZOI-3.75± 0.15) followed by *Escherichia coli* (ZOI-3.45± 0.49), *Streptomyces* (ZOI-3.25± 0.15), *Staphylococcus aureus* (ZOI-3.4± 0.1), and *Bacillus subtilis* (3.05± 0.15) which is not higher as compared of control. Chloroform extracts of *B.lata* stem showing maximum zone of inhibition (3.25± 0.15) against *Bacillus subtilis* followed by *Escherichia coli* (ZOI-3.05± 0.15) and *Staphylococcus aureus* (ZOI-2.7±0.2),which is a reduced amount as compared to control.*Brachiariadistichophylla* leaves are used on the wound for healing. Antibacterial analysis of *Brachiariadistichophylla* shows activity against *Pseudomonas aeruginosa* and *Escherichia coli* (Adamu *et al.,* 2005).

Antifungal activity of Leaf extracts of *B.lata*shown inTable-3. Methanol and chloroform extracts of *B.lata*leaves notshown antifungal activity except against *Candida albicans* (ZOI-3.25± 0.05) compared to control (ZOI-3.8± 0.1). In addition to Antifungal activity of methanol and chloroform extracts of *B.lata*stem not having antifungal activity except against *Aspergillus niger*(ZOI-3.65±0.05) and *Candida albicans* (ZOI-2.45±0.49) as compared to control (Table-4).

Table1: Antibacterial activity of Leaf extracts of *B.lata*

Microorganism	Control ZOI (cm) ±SD	Methanol Extracts ZOI (cm) ±SD)	Chloroform Extracts ZOI (cm) ±SD)
Bacillus subtilis	4.3± 0.1	3.4± 0.1	2.35±0.05
Streptococcus pyogenes	4.2± 0.05	3.4± 0.1	3.2±0.1
Escherichia coli	4.65± 0.25	3.05±0.15	2.65±0.55
Staphylococcus aureus	4.9± 0.1	3± 0.1	0
Streptomyces	4.8± 0.1	0	0

Values are the mean of two replicates, mean ± SD where (n = 2)

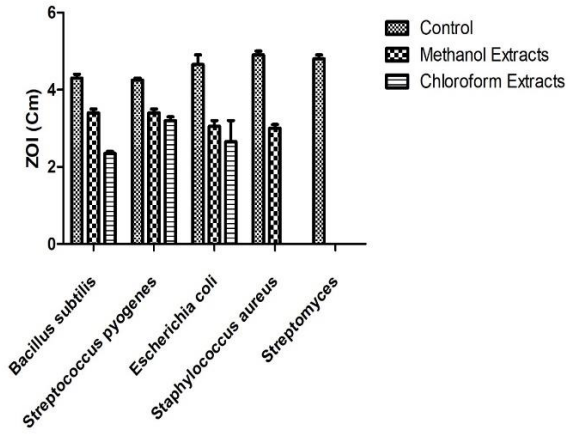

Figure1: Antibacterial activity of Leaf extracts of *B.lata*

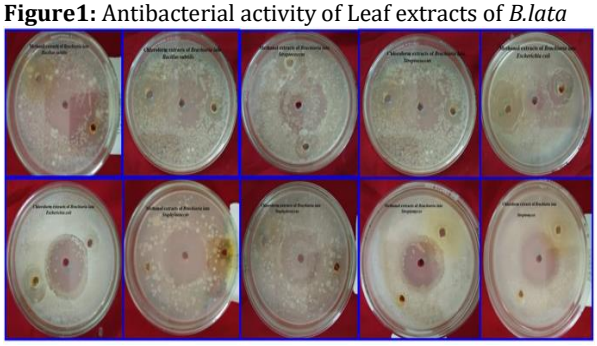

Figure2: Antibacterial activity of Leaf extracts of *B.lata*

Table2: Antibacterial activity of stem extracts of *B.lata*

Microorganism	Control ZOI (cm) ±SD	Methanol Extracts ZOI (cm) ±SD)	Chloroform Extracts ZOI (cm) ±SD)
Bacillus subtilis	4.5± 0.4	3.05± 0.15	3.25± 0.15
Streptococcus pyogenes	4.55± 0.35	3.75± 0.15	0
Escherichia coli	4.35± 0.15	3.45± 0.49	3.05± 0.15
Staphylococcus aureus	5.30.2	3.4± 0.1	2.7±0.2
Streptomyces	5± 0.19	3.25± 0.15	0

Values are mean of two replicates, mean ± SD where (n = 2)

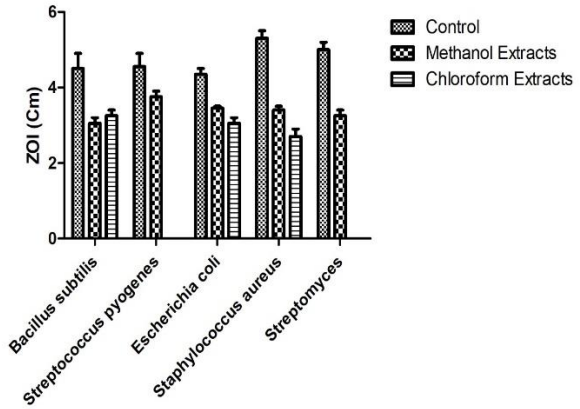

Figure3: Antibacterial activity of stem extracts of *B.lata*

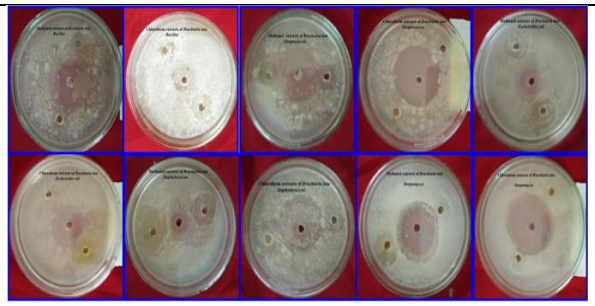

Figure4: Antibacterial activity of stem extracts of *B.lata*

Table3: Antifungal activity of Leaf extracts of *B.lata*

Microorganism	Control ZOI (cm) ±SD	Methanol Extracts ZOI (cm) ±SD)	Chloroform Extracts ZOI (cm) ±SD)
Penicillium chrysogenum	3.7±0.2	0	0
Aspergillus niger	3.95±0.15	0	0
Fusarium oxysporum	3.4±0.2	0	0
Candida albicans	3.8± 0.1	3.25± 0.05	0

Values are mean of two replicates, mean ± SD where (n = 2)

Table4: Antifungal activity of stem extracts of *B.lata*

Microorganism	Control ZOI (cm) ±SD	Methanol Extracts ZOI (cm) ±SD)	Chloroform Extracts ZOI (cm) ±SD)
Penicillium chrysogenum	3.45±0.05	0	0
Aspergillus niger	3.8±0.1	3.65±0.05	0
Fusarium oxysporum	3.65±0.35	0	0
Candida albicans	2.65±0.15	2.45±0.49	0

Values are mean of two replicates, mean ± SD where (n = 2)

Conclusion

We have concluded that the extract of methanol and chloroform extracts of leaves and stem *B. lata*couldbe used to treat bacterial infection. The present study revealedthat the leaves of *B. lata* exploits in the traditional or conventional system of medication to treat various ailments causedby the bacteria. The present outcomes will form the foundation for selection of *B. lata*for further examination in the potential drug discovery of natural bioactive compositesand the isolation and structure elucidation of isolated antibacterial active constituents from the*B. lata*have been started.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Reference

Adamu HM, Abayeh OJ, Agho MO, Abdullahi AL, Uba A, Dukku HU andWufem BM (2005). An ethnobotanical survey of Bauchi State herbal plants and their antimicrobial activity. J. Ethnopharmacol., 99: 1-4.

Agize M, Demissew S and Asfaw Z (2013). Ethnobotany of medicinal plants in Loma and Gena bosa districts (woredas) of dawro zone, southern Ethiopia. Topclass J. Herb. Med.,2: 194-212.

Airy-Shaw HK (1939). Additions to the flora of Borneo and other Malay Islands: XII. The Annonaceae of the Oxford University expedition to Sarawak, 1932. Bull. Misc. Inform.,275-290.

Ajaib M, Khan Z andZikrea A. (2014). Ethnobotanical survey of some important herbaceous plants of District Kotli.Biologia Pak.,60: 11-22.

Amjad MS (2015). Ethnobotanical profiling and floristic diversity of Bana Valley, Kotli (Azad Jammu and Kashmir), Pakistan. Asian Pac. J. Trop. Biomed.,5: 292-299.

Andarge E, Shonga A, Agize M and Tora A (2015). Utilization and conservation of medicinal plants and their associated indigenous knowledge (IK) in Dawuro Zone: An ethnobotanical approach. Int J Medicinal Plant Res., 4: 330-337.

Basappa GP, Muniyamma M andChinnappa CC (1987). An investigation of chromosome numbers in the genus Brachiaria (Poaceae: Paniceae) in relation to morphology and taxonomy. Can J Bot.,65: 2297-2309.

Doss A, Mubarack HM, Vijayasanthi M andVenkataswamy R (2012). In-vitro antibacterial activity of certain wild medicinal plants against bovine mastitis isolated contagious pathogens. Asian J Pharm Clin Res., 5: 90-93.

Hassan A and Ullah H (2019). Antibacterial and antifungal activities of the medicinal plant veronica biloba. J. Chem.,2019: 1-7.

Mitra S and Mukherjee SK (2009). Ethnobotany of some grasses of West Bengal, India. Advances in Plant Biology (Debidas Bhattacharya Birth Centenary Commemorative Volume), 221-253.

Mubarack HM, Doss A, Dhanabalan R andVenkataswamy R (2011). Activity of some selected medicinal plant extracts against bovine mastitis pathogens. J Anim Vet Adv., 10: 738-741.

Nwilene FE, Traore AK, Asidi AN, Sere Y, Onasanya A and Abo ME (2009). New records of insect vectors of rice yellow mottle virus (RYMV) in Cote d'Ivoire, West Africa. J. Entomol., 6: 189-197.

Scorzoni L, Benaducci T, Almeida AMF, SilvaDHS, Bolzani VDS andGianinni MJSM (2007). The use of standard methodology for determination of antifungal activity of natural products against medical yeasts Candida sp and Cryptococcus sp. Braz. J. Microbiol., 38: 391-397.

Shrivastava A and Singh V (2009). Additional floral elements to the Ranthambhore Tiger Reserve, Rajasthan, India. J. Threat. Taxa.,1: 475-480.