Plant Archives Volume 20 No. 2, 2020 pp. 3580-3584 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EVALUATION OF ANTIBACTERIAL POTENTIAL OF AN INVASIVE GRASS (*ARUNDO DONAX* L.) IN A LOWER SIWALIK REGION, INDIA

Aakash Sharma, Sarvjeet Singh, Pardeep Kumar and A.N. Singh*

Department of Botany, Panjab University, Chandigarh-160014 (Punjab) India.

Abstract

Arundo donax L. is a potent invasive weed of aquatic and moist areas distributed worldwide ranging from subtropical low lying hills to warm temperate regions. Despite this, it has a large number of economical applications such as bioenergy production, paper and cellulose production, musical instruments and other household purposes. For medicinal purposes, used as a household remedy for the treatment of various ailments. However, its efficacy against human pathogens is lesser-known. Therefore, we designed to explore in this study to analyze its antibacterial activity against various isolates of bacterial strains. Results indicated that the methanolic root extract showed maximum antibacterial activity against *Pseudomonas aeruginosa* strain (15.0 ± 2.8) at a concentration of 40 ug/ml, while methanolic leaf extract revealed maximum activity against the strain of *Streptococcus aureus* (7.33 ± 3.7) at the same level of concentration. No antibacterial activity was observed against *Bacillus* sp. and *Escherichia coli*. While in the case of methanolic extract of the leaf; *Bacillus* sp. was totally unaffected by the extract concentration. This antibacterial activity can be beneficial by investigating secondary metabolites at ground level for developing new medicines. Therefore, comprehensive studies at a broader scale by considering different concentrations of the extract against several other human pathogens can also be another window to open for exploration on account of harnessing its antibacterial potential.

Key words: *Arundo donax* L., Antibacterial, Disc diffusion, Human pathogens, Medicinal plants.

Introduction

Arundo donax L. belongs to family Poaceae is a potentially high yielding non-food crop, used as production of bioenergy (Lewandowski *et al.*, 2003; Jeon *et al*., 2010; Pilu *et al*., 2012), paper pulp (Perdue, 1958) and wooden building materials. However, it is a robust invasive perennial grass, wildly growing in the southern European regions and other Mediterranean countries (El-Bassam, 1998). It has a high level of adaptability so that can survive into various different ecological conditions mainly in the moist soil and flooded environment.

Invasiveness property of this grass provided ample of distributional scale, therefore, it is found across the globe ranging from subtropical low lying hills to temperate sub-alpine regions across the world. *A. donax* L. is a fairly growing with high density of clump formation evidently reported from the Eastern Asia (Polunin and Huxley, 1987); however, cultivated in many parts of the world such as Asia, Southern Europe, Northern Africa and, the Middle East for a millennium (Perdue, 1958; Oakins, 2001). It is one of the largest members of the genus (*Arundo*) which attains a luxurious growth with height of 6-8 m (Mirza *et al*., 2010). It has a very strong root architecture that penetrates deep into the soil and gives rise to fleshy tuberous rhizomes which spread horizontally and form large thickets. Due to this quality, it is introduced in India generally to reduce soil erosion of the marginal areas of the river, lake, pond and artificial wetlands. Each stem is hollow, cane-like divided into distinct nodes and internodes. Leaf-blades are large somewhat bamboo-like, distichous, linear to lanceolate, rounded or cordate at base, 30-60 cm long and 3-5 cm wide tightly clasped around the stem. The inflorescence is panicle-like 30-60 cm long with the onset of flowering from June-December.

However, the plant is having low palatability as a fodder crop, therefore, little relationships have seen with herbivores. Despite, this has enormous economic value used as a thatching material, in making of musical instruments, paper and pulp production (Perdue, 1958) and source of bioenergy. *Arundo donax* L. is highly desirable for phytoremediation of metal contaminated

***Author for correspondence :** E-mail: dranand1212@gmail.com

sites, owing to its low cost, fast-growth and efficient biomass production. It has the capacity to remediate arsenic from synthetic wastewater bodies (Mirza *et al*., 2010). Moreover, a good soil binder that can reduce soil losses by erosion.

Medicinal and Antibacterial value

Medicinal plants are now getting more attention than ever because they have the potential of myriad benefits to society or indeed to all mankind, especially in the line of medicine and pharmacology. The medicinal value of this plant lies in bioactive phytochemical constituents that produce definite physiological action on the human body (Akinmoladun *et al*., 2007). Fundamentally, antibacterial activity of plants possibly dependent upon secondary metabolites which synthesized into different plant components such as foliage, roots, stem, bark, seeds or seed coats in forms of active principle components like alkaloids, lectins and phenolic compounds such as lactones, tannins flavonoids and alkaloid (Lalitha and Jayanthi, 2012; Al-Rifai *et al*., 2017; Manandhar *et al*., 2019). These secondary metabolites probably function in the protection of seeds from microbial degradation until conditions are favorable for germination (Cai *et al*., 2004; Komutarin *et al*., 2004) and also for protection from herbivores as defense molecules. Also, plant-based remedies are safer and cheaper than synthetic ones (Ruban and Gajalakshmi, 2012; Singh *et al*., 2019).

Ghosal *et al*., (1969) reported various secondary metabolites such as alkaloids (tryptamine, bufotenidine, gramine and arundamine), terpenes and phenols from different parts of *A. donax* L. Plants produce a multitude of organic compounds that have better antibacterial activity. These compounds are found in various plant parts such as stems, roots, leaves, bark, flowers, fruits and seeds and include alliin/allicins, isothiocyanates, plant pigments (Cutter, 2000), hydrolytic enzymes, proteins, essential oils (Smid and Gorris, 1999) and phytoalexins or phenolic compounds (Cutter, 2000).

The rhizome of *A. donax* is a source of various tryptamines *viz*. N, N-Dimethyltryptamine, 5-methoxy-N, N-dimethyltryptamine, bufotenine, etc., which imparts mild psychedelic effects (Ghosal, 1972; Al-Snafi, 2015). Several other bioactive compounds such as N-(4'-bromophenyl)-2,2-diphenylacetanilide, triacontanol, various forms of sterols such as β-sitosterol, stigmasterol, β amyrin acetate, friedelin and campesterol have also been isolated. In India, *Arundo donax* L. is used in several traditional formulations to cure multiple ailments such as a vasopressor, uterine stimulator, hypotensive and antispasmodic agent (Khare, 2008). A study conducted by Kaur *et al*., (2005) reported anti-tumorous properties of a lectin isolated from rhizomes of *A. donax* L. against human cancer cells, however, this finding needs further evaluation. Shirkani *et al*., (2014) demonstrated the antibacterial activity of stem nodes aqueous extract of *Arundo donax* L. against methicillin-resistant *Staphylococcus aureus* (MRSA) at a concentration of 128 μg/ml. Al-Snafi, (2015) reviewed the antibacterial effects of methanolic stem node extracts of *A. donax* against various human pathogens *viz*. *Staphylococcus aureus*, *Micrococcus luteus*, *Klebsiella*, *Escherichia coli* and *Pseudomonas aeruginosa*. However, there is a paucity of information about the antibacterial assay of root and leaf extracts of *A. donax* against human pathogens. Therefore, the present study was undertaken to unravel the antibacterial potential of this grass (*A. donax* L.) for the new medicinal and biocidal purposes.

Material and Methods

Plant material used

In the present study, the antibacterial effect of methanolic leaf and roots extract of *Arundo donax* L. was investigated against various pathogenic strains *viz. Streptococcus aureus*, *Klebsiella pneumoniae*, *Bacillus* sp., *Escherichia coli*, *Pseudomonas aeruginosa*. Leaf and roots from a naturally growing population of *Arundo donax* were collected from Bilaspur and Solan district of Himachal Pradesh (H.P.), India during September - October 2019. The collected specimens were authenticated by consulting Herbarium of Department of Botany Panjab University (PAN), Chandigarh. A voucher specimen of the collected samples has been deposited in the herbarium.

Preparation of plant extract

The collected plant materials were washed thoroughly with distilled water to remove dust and soil particles and shade dried for 10-15 days at room temperature. A fine powder was prepared after grinding of leaves and roots and stored in an airtight container. The methanol extract was prepared by soaking 10 g of dry powdered leaves and roots in 50 ml of methanol. The flask in which extracts were made kept on orbital shaker at temperature 28°C for 48 hours. Then plant extracts were filtered through Whatman filter paper (No. 1) and concentrated using the water bath set at about 37°C. Plant extracts once obtained were preserved at 4°C for future use.

Test organisms and antibacterial activity

The bacterial strains (Clinical isolates) used for antibacterial assay *viz*. *Streptococcus aureus*, *Klebsiella pneumoniae*, *Bacillus* sp., *Escherichia coli*,

Pseudomonas aeruginosa were obtained from Department of Microbiology & Biotechnology, Panjab University, Chandigarh. The antibacterial activity was determined by the disc diffusion method (Bauer *et al*., 1966). Mueller Hinton Agar (38g) with Luria broth (2g) was suspended in 1 litre distilled water and allowed to heat at 121°C (15Lbs) for 15 minutes. After heat, the sterilized Mueller Hinton agar was poured into sterilized Petri plates. After solidification, 100 µl bacterial inoculums adjusted to an OD of 0.8 were swabbed on the respective plates. Whatman No.1 filter paper was punched into 5mm disc and sterilized. Each sterilized disc was incorporated individually with different concentrations of plant extracts using a micropipette. Various concentrations (10 µg/disc, 20 µg/disc, 30 µg/disc and 40 µg/disc) of plant extracts were added to the sterile discs along with positive control of ampicillin in each Petri plate. Then, the plates were incubated overnight at 28°C. After incubation, the diameter of inhibitory zones formed around each disc was measured in mm and recorded.

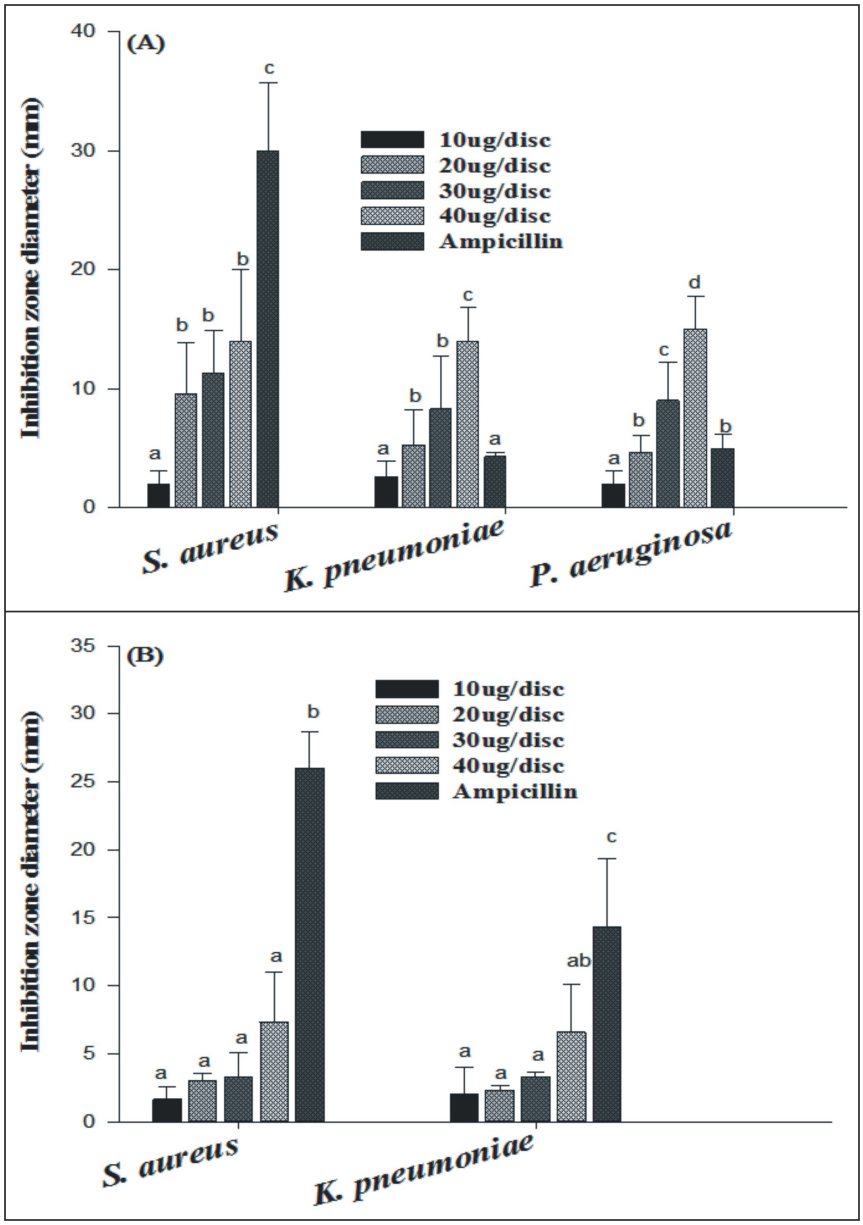

Fig. 1: Efficacy of methanolic root extract (A) and leaf extract (B) of *A. donax* against human pathogens. Values in the bars presented as means of three replicates (n =3) and tips are ± 1SE. Same letters suffixed in the bars are not significantly different in each species within different level of concentrations at $p<0.05$ probability level.

Results and Discussion

The zone of inhibition displayed by methanolic root extract is tabulated in fig. 1a. The methanolic root extract of *A. donax* found most effective against *Pseudomonas aeruginosa* (15.0 ± 2.8) followed by *Klebsiella pneumoniae* (14 ± 2.08) and *Streptococcus aureus* (14.0 ± 6.0) at a concentration of 40 µg/ml. At initial concentration (10 µg/ml) more or less similar effect was observed among all the above discussed bacterial strains. A fairly low sensitivity was seen at 20 µg/ml against *Streptococcus aureus* (9.6±4.3), *Klebsiella pneumoniae* (5.3 ± 2.9) and *Pseudomonas aeruginosa* (4.66 ± 1.45). However, plant extract effectiveness was moderate at 30 µg/ml against *Streptococcus aureus* (11.3 ± 3.6), *Klebsiella pneumoniae* (8.3 ± 4.4) and *Pseudomonas aeruginosa* (4.66 ± 1.45).

The results of the antibacterial activity of methanolic leaf extract are mentioned in fig. 1b. Maximum activity was however observed against *Streptococcus aureus* with maximum inhibition zone (7.33 ± 3.7) at a concentration of 40 µg/ml while minimum inhibition zone against *Klebsiella pneumoniae* (6.6 ± 3.5) at the same concentration. The lowest activity was observed at 10 µg/ml against *Klebsiella pneumoniae* and overall an increasing trend was observed as the concentration of the extract was increased. Sensitivity differences between Gram positive and Gram negative bacteria can be accredited to their morphological variations and permeability of cell wall (Manandhar *et al.*, 2019). No activity

was observed against *Bacillus* sp. and *Escherichia coli*. Lack of activity against some bacterial pathogens may be due to the degradation of bioactive chemicals during drying and extraction processes (Ezeani *et al.,* 2011).

Widespread increase in multidrug resitant pathogens has necessitated to develop plant based antimicrobial therapies (Manandhar *et al.,* 2019). Antibacterial assay of the plant extracts could be attributed to the abundance of different bioactive chemicals (Mummed *et al.,* 2018) *viz*. phenolic and flavonoids. These compounds exert their antimicrobial effect by forming a complex with extracellular and soluble proteins and with the cell wall of bacteria (Ruban and Gajalakshmi, 2012; Rehman *et al.,* 2017). The root methanolic extract has a profound antibacterial assay as compared to leaf methanolic extract. However, the efficacy of ethanolic and chloroform extracts also needs to be investigated in the future to increase our knowledge about the antibacterial potential of *A. donax*.

Conclusion

As far as the medicinal value of *A. donax* is concerned, a wide knowledge gap exists amongst the scientific community because of less exploration of its medicinal properties experimentally. The present study, however, provides first-hand information about the antibacterial potential of root and leaf methanolic extracts of *A. donax* against human pathogens. The methanolic root extract of *A. donax* exhibited maximum efficacy against *Pseudomonas aeruginosa*. However, methanolic leaf extract was highly effective against *Streptococcus aureus*. In order to understand the broad spectrum antibacterial efficacy further studies by taking into account, several other bacterial strains and different concentrations of extracts are therefore recommended.

Acknowledgment

AS and SS would like to thank Dr. A.N. Singh for his valuable guidance, moral support and arousing the interest to initiate this work. Department of Biotechnology, Panjab University Chandigarh is duly acknowledged for providing bacterial strains. Thanks, are also due to the chairperson, Botany Dept., Panjab University, Chandigarh for providing necessary facilities. UGC, Govt. of India is duly acknowledged for providing financial assistance to PK.

References

Akinmoladun, A.C., E.O. Ibukun, E. Afor, E.M. Obuotor and E.O. Farombi (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. *Sci. Res. Essay*., **2:** 163-166.

Al-Rifai, A., A. Aqel, T. Al-Warhi, S.M. Wabaidur, Z.A. Al-Othman and A.Y. Badjah-Hadj-Ahmed (2017). Antibacterial, antioxidant activity of ethanolic plant extracts of some *Convolvulus* species and their DART-ToF-MS profiling. *Evid Based Complement Alternat Med*., **2017**: 1-9.

Al-Snafi, A.E. (2015). Therapeutic properties of medicinal plants: a review of their antibacterial activity. *Int. J. Pharmacol. Toxico*., **6:** 137-158.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, **45:** 493-496.

Cai, Y., Q. Luo, M. Sun and H. Corke (2004). Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci*., **74:** 2157-2184.

Cutter, C. (2000). Antibacterial effect of herb extracts against *Escherichia coli* O 157:H7, *Listeria monocytogenes* and *Salmonella typhimurium* associated with beef. *J. Food Prot.*, **63:** 601-607.

El-Bassam, N. (1998). Energy plant species. Their use and impact on environment and development James & James, London.

Ezeani, M.C., M.I. Agba, C.C. Onyenekwe, I. Anahalu, C.C. Azikiwe and B.E. Unaezeb (2011). Aerobacteriology of laboratories and offices: Evidence of high risk exposure to immune complex formation in Nigeria. *Asian Pac. J. Trop. Dis.*, **1:** 131-136.

Ghosal, S. (1972). Occurrence of psychodelic substances in some Indian medicinal plants. *Planta Med.*, **21:** 200-209.

Ghosal, S., S.K. Dutta, A.K. Sanyal and S.K. Bhattacharya (1969). *Arundo donax* L. (Graminae). Phytochemical and pharmacological evaluation. *J. Med. Chem*., **12:** 480-483.

Jeon, Y.J., Z. Xun and P.L. Rogers (2010). Comparative evaluations of cellulosic raw materials for second generation bioethanol production. *Lett. Appl. Microbiol*., **51:** 518-524.

Kaur, A., J. Singh, S.S. Kamboj, A.K. Sexana and M. Shamugavel (2005). Isolation of an N-acetyl-D-glucosamine specific lectin from the rhizomes of *Arundo donax* with antiproliferative activity. *Phytochemistry.*, **66:** 1933-1940.

Khare, C.P. (2008). *Indian medicinal plants: an illustrated dictionary*. Springer Science & Business Media.

Komutarin, T., S. Azadi, L. Butterworth, D. Keil, B. Chitsomboon, M. Suttajit and B.J. Meade (2004). Extract of the seed coat of *Tamarindus indica* inhibits nitric oxide production by murine macrophages *in vitro* and *in vivo*. *Food Chem Toxicol.*, **42:** 649-658.

Lalitha, T.P. and P. Jayanthi (2012). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Asian J. Plant Sci. Res.*, **2:** 115-122.

Lewandowski, I., J.M.O. Scurloc, E. Lindvall and M. Christou (2003). The development and current status of perennial

rhizomatous grasses as energy crops in the US and Europe. *Biomass Bioenerg.*, **25:** 335-361.

Manandhar, S., S. Luitel and R.K. Dahal (2019). *In vitro* antimicrobial activity of some medicinal plants against human pathogenic bacteria. *J. trop. med.*, **2019:** 1-5.

Mirza, N., Q. Mahmood, A. Pervez, R. Ahmad, R. Farooq, M.M. Shah and M.R. Aziz (2010). Phytoremediation potential of *Arundo donax* in arsenic-contaminated synthetic wastewater. *Bioresour Technol.*, **101:** 5815-5819.

Mummed, B., A. Abraha, T. Feyera, A. Nigusse and S. Assefa (2018). *In vitro* antibacterial activity of selected medicinal plants in the traditional treatment of skin and wound infections in eastern Ethiopia. *Biomed. Res. Int.*, **2018:** 1862401.

Oakins, A.J. (2001). An assessment and management protocol for *Arundo donax* L. in the Salinas Valley Watershed. B.Sc Thesis, California State University, Monterey Bay, USA.

Perdue, R.E. (1958). *Arundo donax* - source of musical reeds and industrial cellulose. *Econ. Bot.*, **12:** 368-404.

Pilu, R., A. Bucci, F.C. Badone and M. Landoni (2012). Giant reed (*Arundo donax* L.): A weed plant or a promising energy crop. *Afr. J. Biotechnol.*, **10:** 9163-9174.

Polunin, O. and A. Huxley (1987). Flowers of the Mediterranean 2nd edition. Chatto and Windus, London, 161.

Rehman, M., N. Akhtar and R. Mustafa (2017). Antibacterial and antioxidant potential of stem bark extract of *Bombax ceiba* collected locally from south Punjab area of Pakistan. *Afr. J. Tradit Complement Altern. Med.*, **14:** 9-15.

Ruban, P. and K. Gajalakshmi (2012). *In vitro* antibacterial activity of *Hibiscus rosa–sinensis* flower extract against human pathogens. *Asian Pac. J. Trop. Dis.*, **1:** 399-403.

Shirkani, A., M. Mozaffari and M. Zarei (2014). Antibacterial effects of 14 Medicinal plant speices of Dashti in Bushehr province. *Iran South. Med J.*, **17:** 49-57.

Singh, S., A. Gupta, A. Kumari and R. Verma (2019). Antimicrobial and antioxidant potential of *Hibiscus rosa-sinensis* L. in Western Himalaya. *Bio. Forum.*, **11:** 35-40.

Smid, E.J. and G. Gorris (1999). Natural antibacterials for food preservation. *Food Science and Technology-New York-Marcel Dekker.*, **4:** 285- 308.