Pak. J. Weed Sci. Res., 19(3): 295 - 303, 2013

BIO-CONTROL ASSAYS OF PLANT PATHOGENIC BACTERIA WITH DIFFERENT SOLVENTS OF *Datura alba* NEES.

Amna Ali[1*], Muhammad Saleem Haider and Sana Hanif

ABSTRACT

The use of plants for the treatment of various human diseases is an old practice and it still offers an enormous potential source of new anti-infective agents. Datura alba Nees (Solanaccae) is popular all over the world for its medicinal uses; hence this study was evaluated for its scientific validity. The antagonistic effect of water and organic solvents (Benzene, Chloroform and Ether) extracts of D. alba were studied against 10 pathogenic strains of bacteria. Among the 10 species, most were shown to have significant anti-bacterial activity with all of the three used extracts. Organic solvent extracts showed anti-bacterial effects towards Xanthomonas axonopodis, Pseudomonas syringae, Enterobacter agglomerans, Clostridium difficile, Ensifer adhaerens and water solvent extracts showed antibacterial effect towards E. agglomerans, Corynebacterium minutissium, Acidovorax temperans, Bordetella pertussis. The minimum inhibitory concentration value for bacterial pathogen ranged between 0.10 to 4.0 mg mL^{-1} when tested with all the solvent extracts of D. alba. The main objective of the study was minimizing the growth of bacterial species by using different extracts/solvents of D. alba. Thus results of D. alba leaf extracts showed effective antibacterial activity with different strains of bacteria, which showed that these are confined to cure the same bacterial diseases.

Key words: Bacteria, *Datura alba*, inhibitory effects, organic and water solvent.

INTRODUCTION

The use of plants and plant products as bio-control agent could be traced as far back the beginning of human civilization. There has been a revival of interests in herbal bio-chemicals. This is due to the increased awareness of the limited ability of synthetic chemical products to treat major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom.

[1]Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus, Lahore, 54590, Pakistan
[*]Corresponding author's email: write2amna@gmail.com

Plants are the basic source of knowledge of modern herbicide. This increasing worldwide interest in plants reflects recognition of the validity of many traditional claims regarding the value of natural products in plant care. A large portion of the world population, especially in developing countries depends on the traditional system of medicines for a variety of plant diseases. Several hundred plant genera are used medicinally and plants are vital sources for potent and powerful drugs (Lewis and Ausubel, 2006; Davidson *et al.,* 2005). Plants contain a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, of the phytochemical constituents and found *in vitro* to have antimicrobial properties. Many spices and herbs used today have been valued for their antimicrobial effects and medicinal powers in addition their flavor and fragrance qualities.

Datura is is a shrub-like perennial herb and containing 15 species, of which *D. alba* was found an important drug plants which is cultivated worldwide for its chemical and ornamental properties. The whole plant has medicinal value, but leaves and seeds alone are recognized as official. It has been used extensively in medicine, as an anaesthetic for setting bones, treating bruises and wounds, skin ulcers, hemorrhoids, asthma, rheumatism, whooping cough, muscle spasm, sciatica etc. The purpose of this study was to investigate the anti-bacterial activity of water and solvent extracts from *D. alba* leaves using the well-diffusion method to assay the susceptibilities of plant pathogenic bacteria.

MATERIALS AND METHODS

Collection of plant materials

Fresh leaves of *D. alba* were collected from various places at University of the Punjab, Lahore, Pakistan. All the collected leaves were washed using tap water and shade dried for three weeks. The dried leaves were then homogenized by using a mixer grinder to make fine powder and were then extracted (Vaidya *et al.*, 2009).

Procurement of pathogenic bacterial strains

A total of ten pathogenic bacterial strains, *Xanthomonas axonopodis* (FCBP 007), *Pseudomonas syringae* (FCBP 010), *Xenorhabdus luminescens* (FCBP 119), *Enterobacter agglomerans* (FCBP 120), *Kurthia gibsonii* (FCBP 225), *Corynebacterium minutissium* (FCBP 137), *Clostridium difficile* (FCBP 138), *Acidovorax temperans* (FCBP 227), *Bordetella pertussis* (FCBP 333) and *Ensifer adhaerens* (FCBP 335) used for this study were obtained from FCBP (Table-1) and their cultures were revived on N.A agar media at 37±2 ^{o}C.

Preparation of water solvent

In water solvent extraction, 500 g of shade dried *D. alba* leaf powder soaked in 2500 mL distilled water for 3 days, squeezed and filtered with the help of cotton cloth. Water content of this filtrate evaporated till the solution in semisolid form. Semi solid solution poured in Petri dish and kept in desiccator contained silica gel for residual water absorption from the extract. The extract was preserved aseptically in a brown bottle at 4 ºC until further use (Vaidya *et al*., 2008).

Preparation of organic solvents

For organic solvent extract, 25 g shed dried powder of *D. alba* was taken and placed in soxhlet glass tube, poured organic solvent (benzene, chloroform and ether, separately) and run the soxhlet apparatus. This mixture of extract evaporated and dried with the help of rotary vacuum evaporator and placed in desiccator for residual absorption of water from extract. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4^{o}C in airtight bottles until further use (Vaidya *et al*., 2008).

Working concentration of solvents

Working concentration of solvents were prepared in sterile 100% dimethylsulfoxide (DMSO) with ratio of 1:1 and used for antibacterial activity assay. Consequently, in negative control sterile 100% DMSO and in positive control Penicillin (5µg/disc) were used against all bacterial pathogens.

Media use for antibacterial activity

Antibacterial activity was checked on Nutrient agar (NA) medium (Difco) (Peptone: 5.0 g; Beef extract: 3.0 g, and Agar: 15.0 g (pH 7.4) g/L). Afterward sterilization was done by autoclaving at 15 lb. pressure for 15 minutes and plates were prepared.

Antibacterial activity assay

Antibacterial activity of water and organic solvents (benzene, chloroform and ether) of *D. alba* leaf were determined by well-diffusion method on NA medium (Mushatq *et al.,* 2012). Well were created in the NA plate using sterile cork borer (8.0 mm) and inoculums containing 10^6 CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 60 µl each of all the above mentioned solvents were poured in the boreholes of the inoculated plates. Penicillin (5 µg/disc) was used as positive control and DMSO as a negative control. The plates were incubated for 24 hrs. at 37ºC temperature and anti-bacterial activity was evaluated by qualifying inhibition zones (IZ) of bacterial growth if any around the wells and were measured (cm). The entire anti-bacterial assay was carried out under strict aseptic conditions. Each treatment was replicated three times as well as repeated twice against each of the

test bacterium. The minimum inhibitory concentration (MIC), which was determined as the lowest concentration of leaf extracts inhibiting the growth of bacterial species, was determined based on the readings.

Statistical analysis

Data were presented in tables and prepared using the excel spread sheet. The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SE of three replicates.

RESULTS AND DISCUSSION

The bacterial infections are considered to be the major cause of mortality in agriculture. Because of the growing bacterial resistance against commercial standard and reserve antibiotics, the search for new active substances with antibacterial activity against pathogenic bacteria is gaining importance (Rattanachaikunsopon and Phumkhachorn, 2010). Several studies have been carried out on antimicrobial activity against bacteria and fungi. Hence the present study was an attempt to find out the potential effect of weed species against bacterial and fungal plant pathogens. Because of the side effects and the resistance that pathogenic micro-organisms built against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of world (Arumugam *et al.,* 2009). Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Plants are employed as important source of medication in many traditional medications (Neves *et al.,* 2009). Continued further exploration of plant-derived antimicrobials is needed today. It has also been widely observed and accepted that the medicinal value of plants lies in the bioactive phytocomponents present in the plants. The water and organic solvents (benzene, chloroform and ether) extracted from *D. alba* leaves gives varied results. Table-1, 2 and Fig. 1 show the results of antibacterial activity and inhibition zone of leaf extracts against pathogenic bacterial strains.

Anti-bacterial activity against water solvent

The water solvent shows comparatively low anti-bacterial activity than the organic solvents on N.A. medium. *E. agglomerans, C. minutissium* and *B. pertussis* possess high resistance ranging from 3.0 to 3.4 cm. Moreover, the growth of *X. axonopodis*, *P. syringae*, *X. luminescens*, *K. gibsonii*, and *A. temperans* was moderately inhibited ranging from 2.0 to 2.5 cm. On the other hand, water solvent showed

the lowest inhibition for *C. difficile* (1.5 cm) and for *E. adhaerens* (1.9 cm) (Table-1, Fig. 1).

Antibacterial activity against organic solvents

The antibacterial efficacy of three solvent extracts of *D. alba* against pathogenic bacteria showed varied level of inhibition. Among treatments, maximum *in vitro* inhibition of tested bacteria *X. axonopodis*, *C. difficile* and *E. adhaerens* was scored in benzene extract which offered inhibition zone of 4.0, 4.5 and 4.0 cm respectively. Further, chloroform extract was effective against *P. syringae* (4.0 cm), *E. agglomerans* (4.0 cm), *C. difficile* (4.0 cm) and *E. adhaerens* (3.7 cm) which recorded significant inhibition zone. Consequently in ether extract, a significant inhibition zone of bacteria *P. syringae*, *C. difficile* and *E. adhaerens* were exhibited 3.7, 3.8 and 3.5 cm of inhibition zone, which was followed by 3.9 cm of *X. axonopodis* and *E. agglomerans* inhibition from ether extract of *L. camara*. However, chloroform and ether extracts were found to be insignificant against *X. luminescens*, *K. gibsonii* and *B. pertussis*. Nevertheless *C. minutissium* (2.0 cm) and *B. pertussis* (2.5 cm) showed poor inhibition in the case of benzene solvent. Despite the fact that *A. temperans* was moderately control by all three solvent extracts (Table-1, Fig. 1).

Minimum inhibitory concentration (MIC)

Datura alba extract in chloroform showed MIC of 2.0 mg mL^{-1} against *X*. *axonopodis, P*. *syringae and E*. *adhaerens* whereas 1.0 mg mL^{-1} for *E. agglomerans* and *C*. *difficile*. Although benzene leaf extract showed MIC of 4.0 mg mL^{-1} against all tested bacteria. Furthermore, ether extract showed MIC of 4.0 mg mL^{-1} against *X*. *axonopodis* and *E*. *adhaerens* while MIC of 2.0 mg mL^{-1} was found against *P*. *syringae* and *E*. *agglomerans* both (Table-2). The MIC of 1.0 mg mL^{-1} was found against *C*. *difficile* bacteria when ether extract was used. Besides, the range of MIC from 8.0 to 10.0 mg mL^{-1} exhibited in control condition. Saranraj *et al*. (2010) found the ethanol extract from *Acalypha indica* leaves comparatively more inhibitory against the human pathogenic bacteria than the ethyl acetate extract. Some reports showed methanol extract with higher antibacterial activity than n-hexane and ethyl acetate (Sastry and Rao, 1994) and some reports showed chloroform to be a better inhibitor of than methanol and benzene (Febles *et al*., 1995).

It is clear that using organic solvents provides a higher efficiency in extracting compounds for antimicrobial activities compared to water based method (Lima-Filo *et al*., 2002). The preliminary results obtained from the water and organic solvent extracts indicate that further investigation and screening is worthwhile. This antibacterial effect depends on solvents which are used to extract

the plant extract. Extract obtained from organic solvents and water solvent shows the different antibacterial properties with same bacterial strains. Those bacterial strains inhibited their growth with organic solvent extract could not inhibited by the water solvent extract. This depends on the presence of polar non polar bioactive or inhibitory compounds on the extract. Organic solvent extracted more concentration of non-polar bioactive compounds from the plant powder and water solvent extracted more concentration of polar bioactive compounds (Vaidya *et al*., 2009). So, presence of different concentration of polar and non-polar compounds on the extract showed different inhibitory effect towards same bacterial strains. In other way water solvent extracted more gram of extract that is mainly polar which inhibited the inhibitory effect of non polar compounds this is also vice versa. The present observation clearly suggested that plants and different microbial antagonists used as biocontrol agents can release many different compounds into their surrounding environment, which results in suppression of disease causing organisms and better growth of plants. This study demonstrates that *D*. *alba* possess antibacterial activities and these findings justify the traditional use of *D*. *alba* in phyto-medicine. In the modern world multiple drug resistance has developed against many microbial infections due to the indiscriminate use of commercial antimicrobial chemicals commonly used in the disease management of crops. Besides, Khan *et al*. (2012) reported that herbicide dose can be reduced in combination with allelopathic crop water extracts and growth inhibition of plant pathogens also control due to allelochemicals.

In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within this plant and also to determine their full spectrum of efficacy. Thus present experiment demonstrates that the leaf extracts of *D*. *alba* exhibit antibacterial effect, which offers a scientific basis for using this weed as a good sources of biocontrol agent against plant diseases. Further work is however required to be done for its formulation by phytochemical and pharmacological studies to discover new antibiotic drugs.

CONCLUSION

In Pakistan, *Datura alba* is an exotic unwanted weedy shrub. *Datura* is now a major weed in many regions and it is regarded as one of the most serious weeds in plantation crops. Although, innumerable biologically active compounds are found in it that possess antibacterial properties. From these results, it can be concluded that *D. alba* extracts showed effective antibacterial activity towards pathogenic bacterial strains. So it's extract useful and effective towards the control of bacterial diseases in plants**.**

Table-1. The ant-bacterial activity of water and organic solvents of *Datura alba* leaf

FCB P #	Pathogenic Bacterial Strains	Source	Zone of inhibition (cm)				
			Control	Water Solvent	Organic Solvents		
					Benzene	Chloroform	Ether
007	*Xanthomonas axonopodis*	*Citrus sinensis* fruit	0.9±0.01 c	2.0±0.05 c	4.0±0.06 a	3.5±0.09 b	3.9±0.08 a
010	*Pseudomonas syringae*	*Pyrus malus* fruit	1.0±0.02 b	2.2±0.04 bc	3.5±0.04 b	4.0±0.05 a	3.7±0.09 a
119	*Xenorhabdus luminescens*	*Citrus sinensis* fruit	0.9±0.01 c	2.4±0.08 b	2.6±0.04 c	2.3±0.06 c	2.7±0.03 bc
120	*Enterobacter agglomerans*	*Cucumis sativus* leaf	0.9±0.01 c	3.0±1.08 a	3.7±0.05 b	4.0±0.09 a	3.9±0.01 a
225	*Kurthia gibsonii*	*Citrus sinensis* fruit	1.2±0.05 a	2.1±0.02 bc	2.9±0.05 bc	2.5±1.05 c	1.9±0.09 d
137	*Corynebacterium minutissium*	*Mentha* spp. leaf	0.9±0.01 c	3.4±1.01 a	2.0±0.08 d	3.0±0.09 bc	2.4±0.04 bc
138	*Clostridium difficile*	*Mentha* spp. root	0.9±0.01 c	1.5±0.06 d	4.5±0.02 a	4.0±0.09 a	3.8±0.08 a
227	*Acidovorax temperans*	*Citrus sinensis* fruit	1.1±0.03 a	2.5±0.09 b	3.0±0.02 bc	3.6±1.08 b	3.3±0.05 b
333	*Bordetella pertussis*	*Citrus sinensis* fruit	1.0±0.06 b	3.0±0.04 a	2.5±0.03 c	1.8±0.06 d	2.1±0.03 c
335	*Ensifer adhaerens*	*Citrus sinensis* fruit	0.9±0.02 c	1.9±0.02 d	4.0±0.02 a	3.7±0.05 b	3.5±0.09 b

Table-2. The minimum inhibitory concentration of solvent extracts of *D. alba* for anti-bacterial activity

Solvent Extracts	Minimum inhibitory concentration mg mL^{-1}				
	X. axonopodis	*P. syringae*	*E. agglomerans*	*C. difficile*	*E. adhaerens*
Benzene	4.00	4.00	4.00	4.00	4.00
Chloroform	2.00	2.00	1.00	1.00	2.00
Ether	4.00	2.00	2.00	1.00	4.00
Penicillin	8.00	8.00	8.00	10.00	9.00

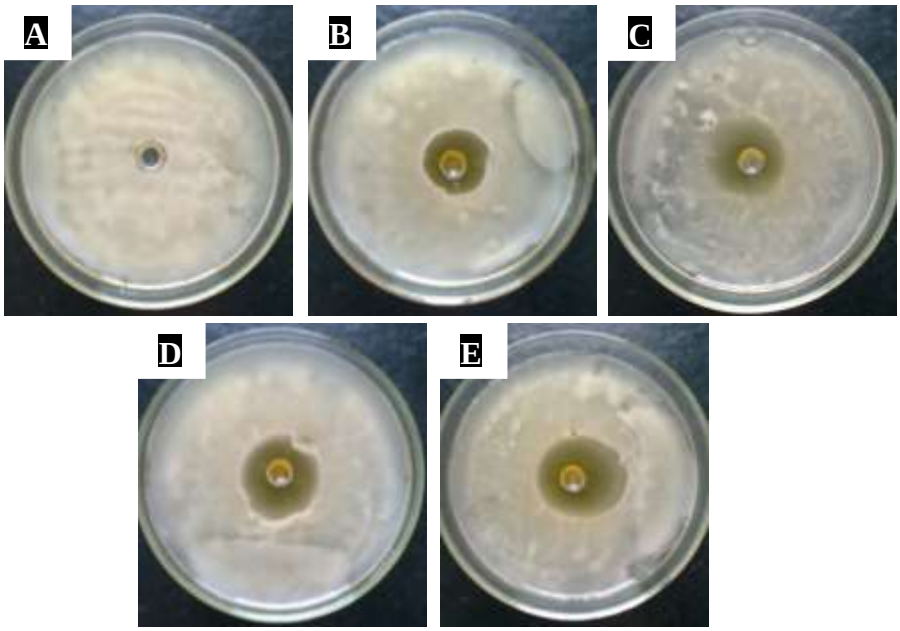

Figure 1. Zone of antibacterial inhibition of different solvents of *D. alba* leaves A. Control (DMSO) B. *E. agglomerans* (3.0 cm) against water C. *X. axonopodis* (3.9 cm) against ether D. *P. syringae* (4.0 cm) against chloroform E. *C. difficile* (4.5 cm) against benzene

REFERENCES CITED

Arumugam, M., S. Karthikeayan and J. Ahmed. 2009. Antibacterial activity of *Indonessiella echioides*. Res. J. Biol. Sci. 1(3): 157-161.

Davidson, P.M., J.N. Sofos and A.L. Branen. 2005. Antimicrobials in food. 3 ed. CRC Press, Taylor and Francis Group. Boca Raton FL 33431, USA.

Febles, C.L., A. Arias and M.C. Gil-Rodriguez. 1995. *In vitro* study of antimicrobial activity in algae (Chlorophyta, Phaeophyta and Rhodophyta) collected from the coast of Tenerife (in Spanish). Anuria del Estudios Canarios. 34: 181-192.

Khan, A., I. Awan, M. Mansoor, E. Khan, A. Khakwani, M.S. Baloch and N. Khan. 2012. Use of concentrated aqueous plant exudates as weed control measure in wheat crop. Pak. J. Weed Sci. Res. 18(1): 99-105.

Lewis, K. and F.M. Ausubel. 2006. Prospects of plant derived antibacterial. Nat. Biotechnol. 24: 1504-1507.

Lima-Filo, J.V.M., A.F. Carvola and S.M. Freitas. 2002. Antibacterial activity of extract of six macro algae from the North eastern Brazilian Coast. Brazilian J. Microbiol. 33: 311-313.

Mushatq, S., S. Haider, A. Ali, S. Javed, I. Khokhar and I. Mukhtar. 2012. *In vitro* Comparative Screening of Antibacterial and Antifungal Activities of Some Common Weeds Extracts. Pak. J. Weed Sci. Res. 18(1): 15–25.

Neves, J.M., C. Matos, C. Moutinho, G. Queiroz and L.R. Gomes. 2009. Ethno-pharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J. Ethnopharmacol. 124(2): 270-283.

Rattanachaikunsopon, P and P. Phumkhachorn. 2010. Potential of cinnamon (*Cinnamomum verum*) oil to control *Streptococcus iniae* infection in tilapia (*Oreochromis niloticus*). Fish Sci. 76: 287-293.

Saranraj, P., D. Stella and S. Samuel. 2010. Antibacterial potentiality of ethanol and ethyl acetate extract of *Acalypha indica* against human pathogenic bacteria. J. Ecobiotechnol. 2(7): 23 -27.

Sastry, S. and G. Rao. 1994. Antibacterial substance from marine algae: Successive extraction using benzene, chloroform and methanol. Bot. Mar. 37: 357-360.

Vaidya, S., K. Shrestha and H. Wallander. 2009. Evaluation of antimicrobial activity of *Lantana camara* Linn. against pathogenic fungi and bacteria. ECOS Proc. 2009.

Vaidya, S., K. Shrestha, B. Khadgi, N. Johnson and H. Wallander. 2008. Organic matter stimulates arbuscular mycorrhizal fungi in Bauhinia purpurea and Leucaena diversifolia plantations on eroded slopes in Nepal. Restor. Ecol. 16(1): 79–87.