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BIOASSAY OF *Nycthanthes arbor-tristis* EXTRACTS AGAINST BACTERIAL AND FUNGAL PHYTOPATHOGENS

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ABSTRACT

Plants are important sources of antimicrobial compounds which since centuries have contributed to control animal and human diseases. In the present study ethanol extracts and fractions of Nycthantis arbor-tristis is tested for its antifungal and antibacterial potential in a series of in vitro experiments. The plant extracts were tested against 5 pathogenic bacteria i.e. (Erwinia carotovora subspecies carotovora, Erwinia carotovora subspecies atroseptica, Clavibacter michiganensis subspecies michiganensis, Ralstonia solanocerum and Erwinia chrysenthemi) and 4 pathogenic fungal stains i.e. (Penicillium notatum, Aspergillus niger, Fusarium solani and Alterneria solani). From the dry leaves and bark different extracts were prepared in solvents of different polarities (ethyl acetate, chloroform, n- hexane and water). Results were recorded as percent growth inhibition, compared with positive control. The results exhibited that ethyl acetate, chloroform and hexane fractions show excellent antifungal activity against the test fungal stains. However, against phytopathogenic bacterial strains no or negligible activity was recorded for all fractions including the crude extract of leaves and bark. Among the extracts tested, crude extract of N. arbor-tristis showed minimum activity against both fungi and bacteria. These in vitro experiments exhibit that in comparison to bacterial pathogens the extract of N. arbor-tristis can be more effective against fungal pathogens.

Key words: Antifungal, Antibacterial, *Nycthantis arbor-tristis*, plant extract, plant pathogens.

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INTRODUCTION

Many disease causing pathogens cause diseases in plants by altering the physiology and organization of cells or tissues (Agrios, 2005). Plant diseases not only decrease crop production but also reduce the economic value of plants. Plant diseases not only affect plant health but their influence can be noticed on human civilization and economy. For example the great Irish Famine due to which many people died and millions migrated was due to a plant fungal disease by *Phythopthora infestans* (Ó Gnoráda, 2004). According to an estimate about 800 million people are suffering from malnutrition globally and plant diseases are responsible for 10% loss of global food production (Gates *et al.*, 1971). Plant diseases not only affect the economy of a country but it adversely affects the health of animal and humans due to secretion of toxins by pathogens. A great amount of resources are spent annually on development of synthetic fungicides and pesticides to control plant diseases which have posed various environmental and health problems (Fry, 2012).

Most of the plant diseases are of fungal origin and in order to control crop fungal diseases synthetic fungicides are considered the most capable solution. However, most of these synthetic fungicides enter the food chain and drastically effect plant and human health. Because of health hazards and restrictions to the use of synthetic fungicides, researchers are striving to discover natural compounds having fungicidal activity (Tepe *et al.*, 2004). Higher plants have the ability to produce large number of organic compounds in which secondary metabolites have been reported for their antimicrobial activities (Castello *et al.*, 2002). Polysaccharides extracted from plants have been reported to have antimicrobial potential against phytopathogens and they are ecofriendly (Bravin *et al.*, 2006). It is reported that aqueous extracts of shoots of pear exhibited strong activity against different pathogenic microorganisms (Jin and Sato, 2003).

Metabolites isolated from higher plants can be used as candidates for natural fungicides. *Cestrum nocturnum* extracts were reported to inhibit the growth of a number of different plant pathogenic fungal stains that include *Phythopthora capsici*, *Fusarium* solani*, Rhizoctonia solani*, *Botrytis cinerea, Colletotrichum capsici, Sclerotinia sclerotiorum* and *Fusarium oxysporum* (Al-Reza et al., 2010). *Sclerotinia sclerotiorum* is the causative agent of carrot rot, the inhibitor of growth which can be inhibited using *Azadirachta indica (*Neem) and *Zingiber officinale* (Ginger) extracts*.* Ethyl acetate and ethanol fractions of leaf extracts from these plants were tested against different isolates of *S. sclerotiorum* and successful growth inhibition was recorded (Ojaghian *et al*., 2014)*.*

The diseases caused by drug resistant pathogens are a serious problem not only to control human and animal diseases but also to control plant diseases. Efforts are being made to synthesize and discover new compounds that can be used as antimicrobials to control plant and animal diseases (Shlaes, 2004). In the present work extracts of N. arbortistis was tested for their antimicrobial potential against various plant pathogenic fungi i.e. *F. solani, A. solani, A. niger, P. notatum and plant pathogenic bacteria like E. carotovora subspecies carotovora, E. carotovora subspecies atroseptica, C. michiganensis subspecies michiganensis, R. solanocerum* and *E. chrysenthemi.*

MATERIALS AND METHODS

Plant material

Leaves and bark of *N. arbor tristis* were collected and identification of these materials was performed using the expertise and facilities available in Department of Botany University of Peshawar, Khyber Pakhtunkhwa. The plant material after collection was shade dried for 10 days and then stored under appropriate conditions for further processing.

Extraction and Fractionation of metabolites

Using electric grinder, dried plant materials were crushed to powder form. After crushing 500g of leaf and bark material in powder form was soaked separately in ethanol for 7-10 days. Filtration was performed using Whatman filter paper and filtrate was collected in clean flasks. The filtrate was then concentrated in rotary evaporator at 45 °C. From leaves dried crude extract of dark green color was obtained while the crude extract of bark was dark brown in color. The crude extracts were further dried using vacuum and solubility of the crude was checked in solvents of different polarity. Different fractions of the extracts were prepared in solvents like chloroform, water, ethyl acetate and hexane in separating funnel using standard procedure for 30 to 40 min at 90°C. The fractions obtained was further filtered and filtrate obtained was subjected to freeze drying and then stored at 18°C till further use.

Determination of antibacterial activity

Different fractions of *N. arbor-tristis* were screened for antibacterial activity against 5 pathogenic bacterial strains i.e. *E. carotovora* subspecies *carotovora, E. carotovora* subspecies *atroseptica, C. michiganensis* subspecies *michiganensis, R. solanocerum* and *E. chrysenthemi*. Antibacterial activity was performed by agar well diffusion method using nutrient agar medium. Bacteria were inoculated to sterile nutrient broth medium autoclaved at 121 $^{\circ}$C and incubated overnight in order to obtain pure cultures. Sterile nutrient agar plates were prepared and allowed it to solidify at room

temperature. The turbidity of bacterial culture was adjusted using 0.5 McFarland turbidity standard and a lawn of bacteria was made on the nutrient agar plates using a sterile cotton swab. Desired number of wells was made with the help of a sterile cork borer and crude extract and different fractions were dispensed into the well using micropipette. Amoxicillin was used as a drug of choice in this experiment. The bacterial plates were incubated at 37 ^{o}C for 24 hours and zone of inhibition was measured in millimeter. The experiment was performed in triplicate and the mean values were recorded.

Determination of Antifungal activity

The antifungal activity of *N. arbor-tristis* extracts was determined using agar tube dilution method (Waliullah *et al.*, 2011) and Miconazole was used as a drug of choice in this experiment. The antifungal activities were performed against 4 pathogenic fungal stains i.e. (*F. solani, A. solani, A. niger* and *P. notatum*) The extracted compounds were dissolved in DMSO and a stock solution of 24mg/1ml was prepared. Sterile Sabouraud's dextrose Agar (SDA) medium (5ml) was added into each test tube after autoclaving and after mixing with test sample. The test tubes were kept in slanting position for solidification at room temperature. The fungal cultures were then inoculated on the slants containing the test samples. These test tubes were then incubated for 7 days at 28 ^{o}C and growth inhibition data was recorded by comparing the growth in test sample with standard drug. Percent inhibition of fungal growth was determined using the following formula.

$$\%\ inhibition\ of\ fungal\ growth = \frac{linear\ growth\ in\ test\ (mm)}{linear\ growth\ in\ control\ (mm)} X\ 100$$

RESULTS AND DISCUSSION

The extracts of *N. arbor-tristis* were screened for antibacterial and antifungal activities against five pathogenic bacteria like *E. carotovora* subspecies *carotovora, E. carotovora* subspecies *atroseptica, C. michiganensis* subspecies *michiganensis, R. solanocerum* and *E.* chrysenthemi and 4 pathogenic fungal stains i.e. (*F. solani, A. solani, A. niger* and *P. notatum*). Results were recorded as percent growth inhibition, compared with positive control.

Crude ethanolic extract and various fractions of *N. arbortristis* were screened against the test bacterial strains for possible antibacterial activity but the results confirmed that the plant extract has either exhibited negligible or no activity against the test pathogenic bacterial strains. Antimicrobial resistance has become a global problem. Higher degree of antibiotic resistance has been reported not only in human pathogenic bacterial strains but also in bacteria that cause diseases in plants. Different strategies that can

improve the current scenario of drug resistance include advancement in natural product research to find novel antimicrobial compounds and appropriate use of available antimicrobials. Antibiotics and chemotherapeutic agents play very important role in controlling bacterial and fungal diseases but their efficacy depends on judicious use to minimize the incidence of resistance (Fahey, 2005).

The results of antifungal assay of the leaf extract reveal that highest activity was recorded for chloroform fraction against *A. solani*, the growth of which was completely inhibited. Against *A. solani* ethyl acetate fraction of leaf extract showed 85% inhibition, *n*-hexane fraction exhibited 89% inhibition, crude leaf extract showed 62% while aqueous fraction showed 74% inhibition. Against *A. niger*, chloroform fraction showed 60%, leaf crude extract showed 36% inhibition, *n*-hexane fraction showed 24% while ethyl acetate fraction exhibited 34% inhibitory affect. Aqueous fraction only showed 5.4% inhibition towards *A. niger*. Ethyl acetate fraction of leaf was active against *P. notatum* (95% inhibition) but showed less inhibitory activity of 12.5% against *F. solani.* However, the growth of *F. solani* was inhibited to a greater degree by *n*-hexane (84%), leaf crude (73%) and aqueous fraction (72%). Antifungal activity of leaf crude extract and different fractions are given in Fig. 1.

Ethyl acetate fraction of the bark extract exhibited 100% inhibitory effect against *A. solani, P. notatum and F. solani.* However considerable inhibition of 47% was recorded against *A. niger.* The bark crude extract exhibited high activity of 70% and 80% against *A. solani* and *P. notatum* respectively while 52.2% inhibition was observed against *A. niger*. No effect of the bark crude was observed on the growth of *F. solani*. The aqueous fraction of the bark of this plant inhibited the growth of *A. niger, A. solani, P. notatum* and *F. solani* by 20%, 65%, 55% and 82% respectively. The results of antifungal activity of bark extract and fractions are given in Fig. 2.

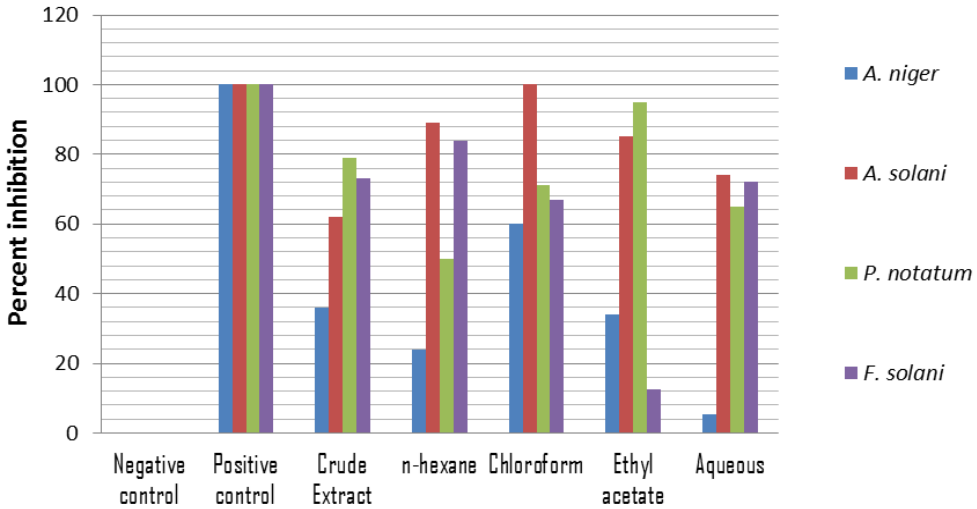

Figure 1. Antifungal activities of leaf extract and fractions

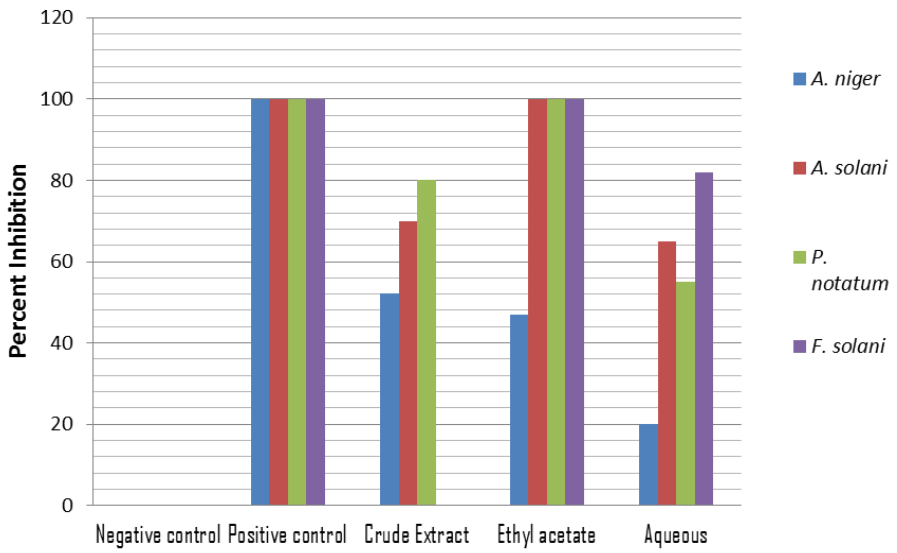

Figure 2. Antifungal activity of bark crude and fractions

Plants are sources of important bioactive compounds which either act as therapeutic agents or provide skeletons for development of novel antimicrobial compounds (Tona *et al.*, 1998). For this purpose a number of plant species have been tested and significant results have been reported. For example plants have been reported to produce compounds with antifungal, antibacterial, anti-viral, anti-inflammatory and antihelminthic properties (Behera and Misra, 2005;

Bylka *et al.*, 2004; Kumarasamy *et al.*, 2002; Palombo and Semple, 2001; Samy and Ignacimuthu, 2000; Stepanović *et al.*, 2003). Research on plant metabolites helps investigate natural products produced by plants, mechanisms responsible for production of these natural products and their potential use as antimicrobials. However, different phytochemicals have been screened against pathogens of humans and animals and less research has been conducted on phytopathogens. Plant metabolites can be used as antibacterial and antifungal agents for control of plant diseases and such metabolites be considered candidates for commercial applications in plant protection.

The *N. arbor tristis* is an ornamental plant commonly known as night jasmine that belongs to Oleaceae family. Apart from its ornamental use this plant has much medicinal values. Different parts of this plant have been screened for antibacterial, antiviral, antifungal and antileishmanial activities (Gupta *et al.*, 2005; Puri *et al.*, 1994; Samy and Ignacimuthu, 2000). In present study leaf and bark extracts were tested against phytopathogenic bacteria and fungi and the results show that ethanol, chloroform and other fractions exhibited signification activity against all fungal strains but no growth inhibitory activities were recorded against bacterial pathogens. Our findings are in accordance to the results of Hirapure and Pote (2014) that tested the bark extract of *N. arbor-tristis* against different plant pathogenic fungi (Hirapure and Pote, 2014). Studies conducted on acetone and aqueous fractions of *N. arbor-tristis* have been documented in which the extracts were tested against pathogenic bacteria. However, no significant antibacterial activity of the extracts was reported (Manisha *et al.*, 2009; Puri *et al.*, 1994; Saxena *et al.*, 2002). Khandelwal (1999) and Manisha *et al.* (2009) reported similar results as no antibacterial activity was observed.

Jain and Singh (2013) also worked on leaf methanol extract of *N. arbor-tristis* and reported significant antibacterial activity against all the test bacterial pathogens. Our results are contradictory to the study reported by Jain and Singh (2013) that tested leaf methanol extract on human pathogenic bacteria but in our study phytopathogenic bacterial strains were used. It is reported by Aggarwal and Goyal (2013) that solvent extracts of different parts of *N. arbor-tristis* show considerable variations in antibacterial activities.

CONCLUSION

The present study concludes that extracts of *N. arbor tristis* are more effective against fungal plant pathogens but has no antibacterial activity against phytopathogenic bacteria. Further investigation may lead to isolation and purification of novel antifungal compounds for use in agriculture.

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REFERENCES CITED

Aggarwal, S. and S. Goyal. 2013. *Nyctanthes arbor-tristis* against pathogenic bacteria. J. Pharmacog. Phytochem. 2(3): 124-127.

Agrios, G. N. 2005. Plant pathology. Amsterdam ; Boston: Elsevier Academic Press.

Al-Reza, S.M., A. Rahman, Y. Ahmed and S.C. Kang. 2010. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum L*. Pestic. Biochem. Physiol. 96(2): 86-92.

Behera, S.K. and M.K. Misra. 2005. Indigenous phytotherapy for genito-urinary diseases used by the Kandha tribe of Orissa, India. J. Ethnopharmacol. 102 (3): 319-325.

Bravin, B., D. Peressini and A. Sensidoni. 2006. Development and application of polysaccharide lipid edible coating to extend shelf-life of dry bakery products. J. Food Eng. 76(3): 280-290.

Bylka, W., M. Szaufer-Hajdrych, I. Matławska and O. Goślińska. 2004. Antimicrobial activity of isocytisoside and extracts of *Aquilegia vulgaris L*. Lett. Appl. Microbio. 39 (1): 93-97.

Castello, M.C., A. Phatak, N. Chandra and M. Sharon. 2002. Antimicrobial activity of crude extracts from plant parts and corresponding calli of *Bixa orellana L*. Indian. J. Exp. Bio. 40(12): 1378-1381.

Fahey, J.W. 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life Journal. 1(5): 1-15.

Fry, W.E. 2012. Principles of plant disease management. Academic Press.

Gates, L., C. McKeen, C. Mortimore, J. Sutton and A. Bolton. 1971. Southern leaf blight of corn in Ontario in 1970. Can. Plant Dis. Surv. 51: 32-37.

Gupta, P., S. Bajpai, K. Chandra, K. Singh and J. Tandon. 2005. Antiviral profile of *Nyctanthes arbortristis L.* against encephalitis causing viruses. Indian. J. Exp. Bio. 43(12): 1156.

Hirapure, P. and M. Pote. 2014. Antimicrobial activity of *Nyctanthes arbor tristis* linn on few clinical isolates. IJPRBS 3(3): 80-85.

Jain, A.K. and K. Singh. 2013. Evaluation of antibacterial studies in harsingar. Int. Res. J. Pharm: 4(7).151-153

Jin, S. and N. Sato. 2003. Benzoquinone, the substance essential for antibacterial activity in aqueous extracts from succulent young shoots of the pear Pyrus spp. Phytochem. 62(1): 101-107.

Khandelwal, K.R. 1999. Antibacterial activity of the leaves of *Nyctanthes arbor-tristis Linn*. Indian. J. Nat. Prod: 18-20.

Kumarasamy, Y., P.J. Cox, M. Jaspars, L. Nahar and S.D. Sarker. 2002. Screening seeds of Scottish plants for antibacterial activity. J. Ethnopharmacol. 83(1): 73-77.

Manisha, V., S. Neha and S. Satish. 2009. Antimicrobial activity of stem bark extracts of *Nyctanthes arbortristis* L. (Oleaceae). IJPPR. 1(1): 12-14.

Ó Gráda, C. 2004. Ireland's Great Famine: an overview.

Ojaghian, M. R., L. Wang, Z. Qi Cui, C. Yang, T. Zhongyun and G.L. Xie. 2014. Antifungal and SAR potential of crude extracts derived from neem and ginger against storage carrot rot caused by *Sclerotinia sclerotiorum*. Ind. Crops. Prod. 55: 130-139.

Palombo, E.A. and S.J. Semple. 2001. Antibacterial activity of traditional Australian medicinal plants. J. Ethnopharmacol. 77(2): 151-157.

Puri, A., R. Saxena, R. Saxena, K. Saxena, V. Srivastava and J. Tandon. 1994. Immunostimulant activity of *Nyctanthes arbor-tristis L*. J. Ethnopharmacol. 42 (1): 31-37.

Samy, R.P. and S. Ignacimuthu. 2000. Antibacterial activity of some folklore medicinal plants used by tribals in Western Ghats of India. J. Ethnopharmacol. 69 (1): 63-71.

Saxena, R., B. Gupta and S. Lata. 2002. Tranquilizing, antihistaminic and purgative activity of *Nyctanthes arbor tristis* leaf extract. J. Ethnopharmacol. 81(3): 321-325.

Shlaes, D.M. 2004. Antibiotic discovery: state of the State. ASM News. 70: 275-281.

Stepanović, S., N. Antić, I. Dakić and M. Švabić-Vlahović. 2003. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol. Res. 158(4): 353-357.

Tepe, B., E. Donmez, M. Unlu, F. Candan, D. Daferera, G. Vardar-Unlu, M. Polissiou and A. Sokmen. 2004. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). Food Chem. 84 (4): 519-525.

Tona, L., K. Kambu, N. Ngimbi, K. Cimanga and A. Vlietinck. 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol. 61 (1): 57-65.

Waliullah, G.U., A. Rauf, B.S. Siddiqui and T.U. Rehman. 2011. Chemical constituents and biological screening of *Grewia optiva Drummond ex Burret* whole plant. American-Eurasian J. Agric. & Environ. Sci.11(4): 542-546.