INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-1, Jan-Mar-2014 Coden : IJABPT Copyrights@ 2014

ISSN : 0976-4550

Received: 23rd Nov-2013 *Revised: 26th Nov-2013* *Accepted: 29th Dec-2013*

Research article

ASSESSMENT OF IN-VITRO ANTI-FUNGAL POTENTIAL OF ETHANOLIC EXTRACT OF *CALLIGONUM COMOSUM* AGAINST TWO FUNGAL POSTHARVEST PATHOGENS OF FRUITS AND VEGETABLES IN SAUDI ARABIA

Jehan Saud Al-Abrahim[1,*], Afrah Eltayeb Mohammed[1] and Mudawi Mukhtar Elobeid[2]

[1] Department of Biology, Faculty of Science, Princess Nora bent Abdul-Rahman University, Postal Code 11474, Riyadh, Saudi Arabia. farhati@hotmail.com

[2] Department of Silviculture, Faculty of Forestry, University of Khartoum, Khartoum north, Postal Code 13314, Shambat – Sudan. emudawi@hotmail.com

* To whom correspondence should be addressed.E-mail: jsaa242@hotmail.com

ABSTACT: The present investigation aimed to evaluate the antifungal activity of *Calligonum comosum* extracts, frequently used in traditional medicine against two phytopathogenic fungi using agar well diffusion technique. *Calligonum comosum* was selected as a model plant species for this investigation on the basis of its reported ethno-botanical uses. Ethanolic extracts of Calligonum comosum plant was screened in vitro for its antifungal activity against two fungal species (Alternaria spp and Rhizopus spp). Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of the extracts were determined. Three extracts were assayed from different parts of the plant (leaves, stem and roots). Generally, the results showed that all extracts inhibited the fungal growth. Regardless of the plant part assayed, the MIC and MFC values of the extracts were in the range between 3.13 and 12.50 mg ml-1 for both fungal species. Interestingly, the highest significant level of mycelia growth inhibition zone was observed in the stem tissues and the lowest level was detected in root tissues. In the current study, the fungal growth inhibition zone was slightly different among the two fungal genera with respect to the plant parts used. In the light of these findings it could be concluded that, the ethanolic extracts of Calligonum comosum exhibit powerful fungicidal properties indicating the presence of potential antifungal compounds effective in the treatment of plant diseases. Further work in no doubt required to identify the compounds in such extracts responsible for the antifungal activity in Calligonum comosum. Provision of detailed information on the chemical constituents of this species will likely open new avenues which help in the development of drugs that could be obtained from this promising medicinal plant species.

Keywords: Antifungal activity, *Calligonum comosum*, minimal inhibitory concentration, minimal fungal concentration, *Alternaria*, *Rhizopus* spp.

INTRODUCTION

Numerous microorganisms cause serious damages to food particularly fruits and vegetables commonly leading to several quality problems related to nutritional value, organoleptic characteristics, and limited shelf life (Agrios 2004). Pathogenic fungi are the main infectious agents in plants, causing alterations during developmental stages including post-harvest but are also indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens (Dellavalle *et al*. 2011). At a global level, it has been reported that over 25% of cereals are contaminated by known mycotoxins produced by fungi which are toxic for both human beings and animals (Satish *et al*. 2007). Rots originated by fungi are among the main causes of postharvest diseases leading to considerable economic losses (Mohamed *et al*. 1996). Fungi such as *Alternaria* spp., *Fusarium* spp., and *Rhizopus* spp. cause serious damage to various fruits and vegetables such as tomatoes (*Lycopersicum esculentum* Mill.) and peppers (*Capsicum annuum* L.), during their postharvest handling (Snowdon 1990; Bautista *et al*. 2000). To address such problems fungicides have been used so frequently, however the many adverse effects of fungicides applications led human to seek environmentally friendly alternatives.

Since long ago plants have been used by human as rich sources of natural products for the purpose of food preservation as well as traditional treatment of many diseases. Plant extracts have been commonly employed successfully in traditional medicine and their contribution regarding health coverage was estimated for over 80% of the world's population, especially in the developing world (WHO 2002). Recently, the acceptability for the medicines from plants has increased greatly not only at the local scale but also worldwide and considered as powerful alternatives to the synthetically conventional drugs because of their rich content of antibiotic prototypes (Srivastava *et al*. 1996; Rabe and Van Staden 1997; Koduru *et al*. 2006). The increasing preference of plant-derived medicines might be on one hand justified by the low cost particularly in the poor countries, and on the other hand due to the fact that medicines from plant origin affect a wide range of antibiotic resistant microorganisms. Moreover, it has become quite evident that there are relatively fewer adverse effects of these natural medicines relative to modern conventional pharmaceuticals (Ozoula *et al*. 2010).

Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves, seeds and flowers possess inhibitory properties against bacteria, fungi and insects (Davicino *et al.* 2007). However, the potential of higher plants as a source of new pharmaceuticals and drugs is still not thoroughly known. Raw drugs are nowadays prepared from different plant parts such as leaves, stem, roots, fruits, flower and twigs via various extraction methods. Some of these raw drugs are collected in little quantities by the local communities to meet their local needs, while commercial raw drugs are usually collected in greater quantities as raw materials for herbal industries (Uniyal *et al*. 2006). In the present study, *Calligonum comosum* L.`Her., "Arta", a member of the family *Polygonaceae* was evaluated for its antimicrobial activity. It is a plant of tropical and subtropical regions with a wide spread in United Arab Emirates and Saudi Arabia. Previous investigations indicated that the ethanolic extract of the aerial parts of *Calligonum comosum* significantly reduced the increase in hind paw oedema induced by carrageenan in rats. Furthermore, a pre-treatment with the extract produced a significant and dose-dependent inhibition to the acute gastric ulcers induced by phenylbutazone, indomethacin (Liu *et al*. 2002). Recently, Elkhalifah (2013) demonstrated that the extract from different plant parts of *Calligonum comosum* exhibited high antimicrobial activity against four pathogenic bacteria. Chemical analysis from previous studies showed that of anthraquinones and flanovonids are the common chemical constituents in Arta. Besides, dehydrodicatechin which had cytotoxic effect and antioxidant activity was also isolated from *Calligonum comosum* when treated with organic solvents (Farid *et al*. 2007; Ghazanfar 1994; Kamil *et al*. 2000). Interestingly, anthraquinones of *Calligonum comosum* showed high antimicrobial potential (Zaki *et al.* 1984). Despite the potential and promising value of *Calligonum comosum* as a medicinal plant, limited knowledge is available regarding its contribution in the treatment of phytopathogens. Thus, this investigation was undertaken mainly to evaluate the antifungal activity in the leaves, stem and roots of *Calligonum comosum* against two phyo-pathogenic fungi.

MATERIALS AND METHODS

Collection of plant material

Calligonum comosum seedlings (38 ± 1.08 cm height) were collected from Dirab experimental farm near Riyadh city, Saudi Arabia. The antimicrobial activity was evaluated in leaves, stem and roots tissues of *Calligonum comosum* against two phytopathogenic fungi.

Preparation of crude extracts

Fresh leaves, stem and roots of *Calligonum comosum* seedlings were cut, washed in distilled water, spread on trays and then air-dried under the sun light. Following drying, the plant materials were mechanically ground to a fine powder by an electrical blender. For the preparation of ethanolic extract, ten grams of the powder was soaked in 100 ml of 90% ethanol for 24 hours under room temperature (22 –25°C). The resultant solutions were filtered through a Whattman filter paper No.1 grade. The filtrate was concentrated through evaporation process using a water bath at 100°C. The extracts were stored in sterile glass bottles at 4°C until use.

Source of pathogens and cultures medium

Two phyto-pathogenic fungi (*Alternaria* spp. and *Rhizopus* spp.) were isolated from tomato fruit and identified in the laboratory of the department of biology – Princess Nora bent Abdul-Rahman University, Riyadh, Saudi Arabia. Potato dextrose agar was used as a growth medium for the fungi investigated in this study.

Assesment of antifungal activity

The antifungal activity was evaluated by noting the zone of inhibition against the tested fungi (Eloff 1988). Two colonies of 24 hours cultured plates were transferred to 10 ml distilled water in test tubes and mixed thoroughly to maintain uniform distribution. A sterile cotton swab was then used to spread the resulting suspension on the potato dextroas agar and allowed to dry for 10 minutes. Subsequently, two adequately spaced wells (holes) of 4 mm diameter each were made per plate at the culture agar surface using sterile metal cup borer. In each hole, 0.2 ml of each extract and control were put under aseptic conditions, kept at room temperature for one hour to allow the agent to diffuse into agar medium and incubated accordingly.

Distilled water was used as negative control. The plates were then incubated for 48 hours at 28°C. At the end of the incubation period the zones of inhibitions were measured to the nearest millimeter (Andrews *et al*. 2001). The inhibition zone is the area surrounding the hole with no growth of inoculated fungi. For confirmation of the results each test was performed in triplicate.

Determination of minimal inhibitory concentration (MIC) of the extract

A microplate method described by Eloff (1998) was used with slight modifications to determine minimal inhibitory concentration (MIC) values of plant extracts. The lowest concentration of the extract that inhibits the growth of the tested microorganisms is conventionally known as the minimal inhibitory concentration. The initial concentration of the plant extract (100mg/ml) was diluted using double fold serial dilution by transferring 5 ml of the sterile plant extract into 5 ml sterile distilled water to obtain the concentration of 50 mg/ml (Oboh *et al.* 2007). Different concentrations were prepared from the crude extracts by doubling dilution in distilled water to get the following concentrations (50, 25, 12.5, 6.25 and 3.13 mg/ml). Each individual dilution was introduced in to nutrient agar plates already seeded with the microorganisms and all plates were incubated for 48 hours at 28°C. Agar plates with the lowest concentration of the extract (without microbial growth) are considered as the minimal inhibitory concentration (Eloff 1988).

Minimal fungicidal concentration (MFC)

The minimal fungicidal concentration (MFC) of the plant extract was determined using the method described by Igbinosa *et al*. (2009). Samples were taken from the plates with no visible growth in the MIC assay and sub-cultured on freshly prepared potato dextrose agar and incubated for 48 hours at 28°C. The MFC was taken as the concentration of the extract that did not show any growth on the new set of agar plates.

Statistical analysis

Data was statistically treated with the statistical programme JMP 5.1 Start Statistics, third edition (SAS Institute, Inc., Cary, North Carolina, USA). Variations among the different treatments were tested using analysis of variance, ANOVA. Results presented are means (3 replicates ± SD). Tukey – test was used for the separation of means. A probability level of $P < 0.05$ was chosen to indicate the significant differences.

RESULTS AND DISCUSSION

Plants are capable of producing secondary metabolites which serve as biological protection against a wide spectrum of harmful microorganisms. These compounds have been obtained from plant extracts for food or medical applications (Wallace 2004). In this study, assessment of the antifungal activity of organic extracts from *C. comosum*, a wild medicinal plant from arid regions of Riyadh, Saudi Arabia was evaluated. In our current investigations results indicated that the activity of ethanolic extracts of *Calligonum comosum* against the two phytopathogenic fungi (*Alternaria* spp. *and Rhizopus* spp.) was different from plant part to another (Table 1). Similar findings on the evaluation of 20 different plant species for their *in-vitro* effect on development of the postharvest phytopathogenic fungi: *Alternaria* spp. *and Rhizopus* spp. were obtained (Bautista-Baños *et al*. 2000). Moreover, Mondali *et al*. (2009) and Dellavalle *et al.* (2011) provided supporting evidence on the positive effect of the inhibiting capacity of plant extract on the mycelia growth of *Alternaria* spp. and *Rhizopus* spp.

Table 1: Zone of inhibition (mm) of ethanolic extract of various parts of *Calligonum comosum* plant on tested fungi. Values are mean ± std of three replicates. Values within a row with different alphabets are significantly different from each other at a probability level of P ≤ 0.05. Means with no inhibition zone detected are indicated as dash (-).

Treatment	Leaves	Stem	Root	Distilled water
Alternaria spp.	14.17±0.99 b	18.00±1.09 a	10.00±0.63 c	-
Rhizopus spp.	15.60±0.89 b	19.70±1.08 a	11.03±0.73 c	-

The extract from the stem tissues showed the highest zone of inhibition for both tested fungi (*Alternaria spp.* 18.00±1.09 mm and *Rhizopus spp*. 19.70±1.08 mm). On the other hand, the extracts from the roots exhibited the lowest inhibition zone for the tested organisms compared to leaves and stem extracts for both tested fungi (Table 1). This result is in well agreement with Elkhalifa (2013) who showed the same pattern of response regarding the treatment of Gram positive and Gram negative bacteria with *Calligonum comosum* regarding root extract.

However, with regard to the antimicrobial activity from the leaf and stem tissues our findings didn't agree with Riadh *et al.* (2011) and Elkhalifa (2013) who investigated *Calligonum comosum* and noted the significant level of antibacterial activity form the leaves tissue compared to stem tissue on the growth of the bacterium, *Listeria ivanovii*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and S*taphylococcus aureus* when laboratory tested by the agar well diffusion method. With regard to the minimal inhibitory concentration (MIC) of plant extracts against the fungal strains it was also varied from plant part extract to another (Table 2).

Table 2: Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) in mg⁄ml of the ethanolic extract of *Calligonum comosum* plant against tested organisms.

Plant parts	Tested organisms			
	Alternaria spp.		*Rhizopus spp.*	
	MIC	MFC	MIC	MFC
Leaves	3.13	6.50	3.13	6.50
Stem	3.13	3.13	3.13	3.13
Root	12.50	12.50	12.50	12.50

The MIC of leaves and stem extracts was 3.13 $mgml^{-1}$ while that of the roots extract was 12.50 $mgml^{-1}$ for both tested fungi. On the other hand, the minimal fungicidal concentration of the ethanolic extract of the leaves was 6.50 $mgml^{-1}$, while that of the stem extract was 3.13 $mgml^{-1}$ and for the roots extract was 12.50 $mgml^{-1}$ (Table 2). Furthermore, results of the present investigation clearly indicated the efficiency of all part of *Calligonum comosum* plant for the suppression of fungal growth. It is worth mentioning that the low MIC was observed to be correlated with the higher antimicrobial activity of the extract as observed in the extracts from the stem and leaves tissues compared to the roots extract (Table 2). In conclusion, our current findings pointed to the ability of the extraction from different parts of *Calligonum comosum* to suppress the growth of phytophathogenic fungi *Alternaria* spp. and *Rhizopus* spp. Song *et al.* (2007) documented that, anthroquinones, terpenoids and flavonoids have positively controlled the growth of dental caries caused by *Streptococci* when treated with separated fraction from *Polygonum cuspidatum*. Seemingly, the inhibiting activity of the ethanolic extract from the different plant parts of *Calligonum comosum* is likely linked to the presence of some special compounds such as anthroquinones, flavonoids and dehydrodicatechin with cytotoxic and antioxidant activity (Ghazanfar 1994, Kamil *et al*. 2000, Farid *et al.* 2007).

CONCLUSIONS

The current results showed that extracts of *Calligonum comosum* medicinal plants exhibited antifungal effects against *Alternaria* spp. and *Rhizopus* spp. In particular, stem ethanolic extracts of *Calligonum comosum* showed growth inhibition of the tested fungi even at low concentrations. Further studies are definitely needed to confirm our findings for the improvement of our knowledge on the potential of the *Calligonum comosum* extract as antimicrobial in relation to its chemical constituents.

REFERENCES

Agrios GN (2004). Losses caused by plant diseases. Plant Pathology. Elsevier, Oxford, UK: 29 – 45.

Andrews JM (2001). Determination of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy 48 (1): 5 – 16.

Bautista-Baños S, Hernández-López M, Barrera-Necha LL (2000). Antifungal screening of plants of the state of Morelos, Mexico against four fungal postharvest pathogens of fruits and vegetables. Revista Mexicana de Fitopatología 18 (1): 36 – 41.

Bautista-Baños S, Hernández-López M, Díaz-Pérez JC, Cano-Ochoa CF (2000). Evaluation of the fungicidal properties of plant extracts to reduce *Rhizopus stolonifer* of 'ciruela' (*Spondias purpurea* L.) during storage. Postharvest Biology and Technology 20: 99 –106.

Davicino R, Mattar MA, Casali YA, Graciela S, Margarita E, Micalizzi B (2007). Antifungal activity of plant extracts used in folk medicine in Argentina. Revista Peruana de Biología 14: 247 – 251.

Dellavalle PD, Cabrera A, Alem D, Larrañaga P, Ferreira F, Rizza MD (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus *Alternaria* spp. Chilean Journal of Agricultural Research 71(2): 231 – 239.

Elkhalifa DHM (2013). In-vitro antibacterial activity of ethanol extract of *Calligonum comosum* plant against four human pathogens in Saudi Arabia. International Journal of Plant, Animal and Environmental Sciences 3 (4): 170 – 175.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica 64 (8): 711 – 713.

Farid AB, Madiha A, Mohamed RA (2007). Evaluation of cytotoxic compounds from *Calligonum comosum* L. growing in Egypt. *Z.* Naturforsch 62c: 656 – 660.

Ghazanfar SA (1994). Handbook of Arabian Medicinal Plants. CRC Press, p.173.

Igbinosa OO, Igbinosa EO, Aiyegoro AO (2009). Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African Journal of Pharmacy and Pharmacology 3 (2): 058 – 062.

Kamil M, Jayaraj AF, Ahmad F, Gunasekhar C, Samuel S, Habiballah M, Chan K (2000). Pharmacognostic and phytochemical standardisation of *Calligonum comosum*. Journal of Pharmacy and Pharmacology 52: 262.

Koduru S, Grierson DS, Afolayan AJ (2006). Antimicrobial activity of *Solanum aculeastrum*. Pharmaceutical Biology 44: 283 – 286.

Liu XM, Zakaria MN, Islam MW, Radhakrishnan R, Ismail A, Chen HB, Chan K, Al-Attas (2002). Anti-inflammatory and anti-ulcer activity of *Calligonum comosum* in rats. Fitoterapia 72 (5): 487 – 491.

Mohamed S, Saka S, El-Sharkawy SH, Ali AM, Muid S (1996). Antimycotic screening of 58 Malaysian plants against plant pathogens. Pesticide Science 47: 259 – 264.

Mondali NK, Mojumdar A, Chatterje SK, Banerjee A, Datta JK, Gupta S (2009). Antifungal activities and chemical characterization of Neem leaf extracts on the growth of some selected fungal species *in-vitro* culture medium. Journal of Applied Science and Environmental Management 13 (1): 49 – 53.

Oboh IE, Akerele JO, Obasuyi O (2007). Antimicrobial activity of the ethanol extract of the aerial parts of *Sida acuta* Burm. (*Malvaceae*). Tropical Journal of Pharmaceutical Research 6 (4): 809 – 813.

Ozoula IR, Idogun SE, Tafamel GE (2010). Acute and sub-acute toxicological assessment of aqueous leaf extract of *Bryophyllum pinnatum* (Lam.) in Sprague-Dawley rats. American Journal of Pharmacology and Toxicology 5 (3): 145 – 151.

Rabe T, Van Staden (1997). Antibacterial activity of South African plants used for medicinal purposes. Journal of Ethnopharmacology 56: 81 – 87.

Riadh H, Imen F, Abdelmajid Z, Sinda F (2011). Detection and extraction of Anti-Listerial compounds from *Calligonum comosum*, a medicinal plant from arid region of Tunisia. African Journal of Traditional Complementary and Alternative Medicine 8 (3): 322 – 327.

Satish S, Mohana DC, Raghavendra MP, Raveesha KA (2007). Antifungal activity of some plants extracts against important seed-borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology 3: 109 – 119.

Snowdon AL (1990). A colour Atlas of postharvest diseases and disorders of fruits and vegetables 1: 302 General Introduction & fruits. Wolfe Scientific Publications, London.

Song JH, Yang TC, Chang KW, Han SK, Yi HK, Jeon JG 2007. In vitro effects of a fraction separated from *Polygonum cuspidatum* root on the viability, in suspension and biofilms, and biofilm formation of mutants streptococci. Journal of Ethnopharmacology 112 (3): 419 – 425.

Srivastava J, Lambert J, Vietmeyer N (1996). Medicinal plants: An expanding role in development. World Bank Technical Paper. No 320.

Uniyal SK, Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. Journal of Ethnobiology and Ethnomedicine 2: 1 – 14.

Wallace RJ (2004). Antimicrobial properties of plant secondary metabolites. Proceedings of Nutrtion Society 63:621-29.

WHO (2002). Traditional medicine: Growing needs and potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva: 1 – 6.

Zaki D, Abd-El-Aziz M, El-Gengeihy S, Morsi N (1984). Antimicrobial potentialities of some Egyptian desert plants. Herba Hung 23: 73 – 84.