IN VITRO SCREENING OF *CIRCIUM ARVENSE* FOR POTENTIAL ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES

ARIFULLAH KHAN*[1], ADNAN AMIN[3], MUHAMMAD AYAZ KHAN[4] AND IRSHAD ALI[2]

[1*,2]*Department of Chemistry, Gomal University, D.I. Khan, Pakistan*

[3, 4]*Gomal Center of Biochemistry and Biotechnology (GCBB) Gomal University, D.I. Khan, Pakistan*

ABSTRACT

The antimicrobial activities of *Circium arvense* extracts were evaluated against two human gram positive (*Staphylococcus aureus, Micrococcus luteus*) and four gram negative pathogen (*Escherichia coli, pseudomonas aeruginosa, Enterobacter, Klebsiella pneumoniae*). The Methanol extracts of *Circium arvense* were used to obtain various fractions (X1) n-hexane, (X2) chloroform, (X3) ethyl acetate and (X4) n-butanol. The agar well diffusion assay and agar dilution susceptibility testing were carried out to determine the zone of inhibitions and the minimum inhibitory concentration respectively. Antifungal activity was employed by agar well diffusion method. The Ampicilline, Ofloxacine and Itraconazole were used as standard agents. Almost all fractions exhibited more or less antimicrobial activity. The X2 (Chloroform) fraction was reported as highly active fraction with good antimicrobial activity MIC 0.312 mg/ml against staphylococcus areous. The MBC values of fraction X2 (chloroform) was 2 times the MIC. Similarly all fractions exhibited good inhibitory activities against *Aspergillus niger*. The fraction X1(n-hexane) was found highly active 6(±0.11), followed by X2 (chloform) fraction 5 (±0.17). Present study provides a base to explore the antimicrobial potential of *Circium arvense*. It is concluded that the *Circium arvense* having potential antibacterial and antifungal activities, irrespective of fact that the MIC values reported were high. Further investigations are recommended to exploit the hidden medicinal value of plant.

Keywords: Traditional medicine, crude extract, antimicrobial activities, minimum inhibitory concentration.

INTRODUCTION

Throughout the history of mankind medicinal plants have continuously been used for the treatment of multiple infections (Augustin and Hoch, 2004; Ashraf *et al*, 2006). The sciences of traditional medicinal plants is practiced successfully worldwide (Anon, 2002) as a significantly wide majority of people rely on herbal medicine (Goleniowski *et al*, 006). In spite of development of novel drugs in modern times to combat emerging infections increased resistance to antibiotics of many bacteria is still s global threat (Konig *et al*, 2000). This provoked researchers to screen plant extracts and plant compounds for antimicrobial agents (Yoshikazu *et al*, 2001; Norton, 2000).

Cirsium arvense (L.) a cirsium specie Scop. (Asteraceae) (Canada thistle) is a native plant Europe, Asia (northern), and widely introduced elsewhere. It is herbaceous plant growing between 30-100 cm, associated with widespread colonies (Morishita, 1999).

Cirsium arvense is reported nearly in all crops including pastures and rangelands. Likewise *Cirsium arvense* is having a role of natural host to various plant pathogens causing crop spoilage (Parendes and Jones, 2000). Livestock tend to dislike and avoid Canada thistle and may also reduce their consumption of desirable plants in the vicinity of Canada thistle colonies (Leininger, 1988) The crude protein, in-vitro digestible dry matter, micro and macromineral concentrations of Canada thistle are comparable to or greater than those of alfalfa (*Medicago sativa*) (Myers, 2000).

Chemotaxonomic evidence plus preliminary chemical data suggests that the phyto-toxic compounds isolated from *C. arvense* are polyacetylenic in nature (Norton, 2000). Similarly tannins, gallic acid (Jolanta *et al*, 2008) taraxasterol, aplotaxene and their derivatives, in addition to flavonoids are present in the plant (Donald, 1994).

The vulnerary use *Cirsium arvense* had not previously been cited in the principal pharmacobotany texts. However *Cirsium arvense* juice of the leaves, locally applied, for healing of wounds is used in some parts of world (Raven and Edwards 2001; Zouhar, 2001). Fewer reports are available to address the antimicrobial potential of *Cirsium arvense* (Maria *et al*, 2010). No reports are available about antifungal activities of this plant to best our knowledge.

The present study was conducted to evaluate antimicrobial and antifungal activity *Circium arvense* used locally as traditional medicine against multiple infections.

**Corresponding author:* e-mail: dani_amin79@yahoo.com

MATERIAL AND METHODS

Plant Material

Collection was based on information given by local inhabitants during follow-up of ethno medical and traditional uses of plants against infectious diseases used locally (Fabricant and Farnsworth, 2001). Plant identification was performed at Faculty of pharmacy Gomal University, D. I. Khan, Pakistan. The specimens were deposited and voucher specimen number was obtained.

Preparation of crude extracts

One hundred grams of each powdered plant material were extracted with 80% methanol by maceration for 48 h with frequent agitation and the resulting liquid was filtered (Whatman No. 3 filter paper, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor (BU¨ CHI Rota-vapor R-205, Switzerland) at not more than 40°C. The aqueous residue was then placed in an oven at 40°C for about 48 h to remove the water. The resulting dried mass was then powdered, packed into a glass vial and stored in a desicator using silica gel (Ieven *et al*, 1979).

Preparation of fractions

About 100 g of Circium arvense were sequentially extracted with n-hexane, chloroform, butanol and methanol using Soxhlet apparatus. The solvent was evaporated under reduced pressure and the fractions were then placed in a vacuum oven at not more than 40°C for about 24 h to remove any residual solvent. The resulting semisolid mass of each fraction was stored in a desicator using silica gel (Ieven *et al*, 1979).

Microorganisms

Six bacterial species, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, *Pseudomonas aeruginosa* (clinical strain/PIMS), *Enterobacter* (clinical strain/PIMS), *Staphylococcus aureus* (MRSA, clinical strain/PIMS) and *Micrococcus luteus* (clinical strain/PIMS) were used in antimicrobial assay. Strains were obtained from Microbiology Research Lab (MRL) Microbiology Department, Quaid-i-Azam University, Islamabad, Pakistan where these were identified and characterized. These strains were maintained on agar slants at 4°C in Gomal Center of Biochemistry and Biotechnology (GCBB) for antimicrobial tests. Microorganisms were incubated overnight at 37°C in Mueller-Hinton Broth (Oxoid) at pH 7.4. The reference antibiotics used were ofloxacin (10µg) and Ampicillin (10µg) (Oxoid) (table 1).

Antimicrobial screening

Screening for antibacterial activity

The antibacterial activity was determined using agar well diffusion method (Hadacek and Greger, 2000). All bacterial cultures were first grown in nutrient broth at 37°C for 18-24 h incubated till turbidity became equivalent to McFarland 0.5 turbidity standard was obtained. The inocula of the respective bacteria were streaked on to the Muller Hinton agar (Oxoid) plates using a sterile swab in order to ensure a uniform thick lawn of growth following incubation. Wells of 6 mm in diameter were formed on to nutrient agar plates using a sterile cork borer. The wells were filled with the test agents (100 µl) and the plates were then allowed to stay for 1-2 hours at room temperature. Finally, the plates were incubated at 37°C for 18-24 h and the resulting diameters of zones of inhibition were measured.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the crude extracts was determined by agar dilution method (EUCAST Definitive Document, 2000; Mukherjee, 2002 ; Anon, 2000). The sterilized Muller Hinton Agar (oxoid) was allowed to cool to 50°C and about 19 ml of this was added to sterilized test tubes which contained 1ml of different concentration of crude extract. This mixture was thoroughly mixed and poured into pre-labeled sterile Petri dishes. Petri dishes having only growth media were prepared in the same way so as to serve for comparison with petriplate containing crude extract. The concentrations of the extracts used in this test ranged from 2000µg to 0.156 µg/ml. The suspensions of the respective microorganisms having density adjusted to 0.5 McFarland turbidity standard were inoculated onto the series of agar plates using standard loop. The plates were then incubated at 37°C for 24 hours. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC. All tests were carried out in triplicate

Minimum Bactericidal Concentration (MBC)

Minimum Bactericidal Concentration (MBC) of the selected plant parts was measured by the viable cell count method (Toda *et al*, 1989, Anon, 2003), and the results were expressed as number of viable cells as a percentage of the control.

Screening for antifungal activity

The required amounts of each fungal strain was suspended in 2ml of sabauraud dextrose broth. This suspension was uniformly spread on Petri plates containing sabauraud dextrose agar media using sterile swabs. Samples were applied into wells using same technique for bacteria and incubated at 25°C for 3 days. The plates were then examined for the presence of zones of inhibition and the results were recorded. Itraconazole was used as a positive control.

RESULTS

Almost all fractions of *Circium arvense* presented encouraging activity against both gram positive and

Table 1: Zone of Inhibitions of reference antibiotics.

Antibiotic	Microorganism/mm [a]					
	Ec	Kp	Ent	Ps	Ml	Sta
Ampicilline	14.1(±0.05)	12.3(±0.5)	14(±0.11)	11(±0)	13(±0.05)	14(±0.05
Ofloxacine	15.1(±0.2)	14.0(±0.1)	13(±0)	11.9(±0.1)	10(±0.11)	9.06(±0.05)

Ec - *E. coli*, Kp - *Klebsiella pneumoniae*, Ent - *Enterobacter*, Ps - *Pseudomonas aeroginosa*, Ml - *Micrococcus luteus*, Sta - *Staphylococcus aureus* (methicilline resistant), mm - Millimeter

Table 2: Inhibition zones of *Circium arvense* plant extracts

Fraction [a]	Zone of Inhibition (mm)					
	Ec	Kp	Ent	Ps	Ml	Sta
X1(n-Hexane)	3.0(0)	na	5(±0.11)	na	7(±0.5)	2(±0.1)
X2(chloroform)	10 (±0.11)	9(±0.05)	14(±0.11)	8(±0.11)	13(±.05)	15(±0.05)
X3 (ethyl acetate)	5(0)	1(0)	5(±0.05)	2(±0.11)	6(±0.11)	na
X4 (butanol)	7(±0.05)	na	6(±0.05)	3(±0.17)	4(±0.11)	na

Ec - *E. coli*, Kp - *Klebsiella pneumoniae*, Ent - *Enterobacter*, Ps - *Pseudomonas aeroginosa*, Ml - *Micrococcus luteus*, Sta - *Staphylococcus aureus* (methicilline resistant). na -not active, [a] mm - millimeter

negative bacterial pathogens. The X2 (chloroform) fraction was reported as most active fraction against all bacterial strains especially, against *Enterobacter*, *Staphylococcus aureus* and *Micrococcus luteus.* Like wise the n-butanol, ethyl acetate and n-hexane fractions exhibited reportable inhibitory effects against bacterial pathogens (table 2). As a result of significantly high antibacterial activity, the X2 fraction was further processed for determination of MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) respectively. The MIC values ranged from 0.312 to >10mg/ml for all tested strains while the MBC values reported were two time higher than MIC (table 3).

Table 3: MIC and MBC *Circium ravense* (X2) plant extract

X2(Chloroform) Fraction	Minimum Inhibitory concentration (mg/ml)	Minimum bactericidal concentration (mg/ml)
Escheria Coli	> 10	N.d
Klebsiella pneumoniae	> 10	N.d
Pseudomonas aeroginosa	>10	N.d
Enterobacter	5	15
Micrococcus luteus,	0.625	1.875
Staphylococcus aureus	0.312	1.24

N.d. - not determined, Mg/ml - milligram per milliliter

Like wise nearly similar pattern of susceptibility was reported against fungal strain *Aspergillus niger*. The widest zones of inhibition (maximum antifungal activity) was presented by X1 (n-hexane) and X2(chloroform) fractions. The X3(ethyl acetate) and X4(n-butanol) fractions showed comparatively lesser zone of inhibitions (table 4).

Table 4: Antifungal activities of *Circium arvense* crude extracts.

Fraction	Zone of Inhibition (mm)[a]
X1 (n-Hexane)	6 (±0.11)
X2(chloroform)	5 (±0.17)
X3(ethyl acetate)	2 (±0.0.17)
X4(butanol)	4(0)
Standard (itraconazole)	8(0)

[a] mm millimeter

DISCUSSIONS

The antimicrobial activities of four crude extracts (n-hexane, chloroform, ethyl acetate, and n-butanol) were tested against six bacterial species Viz *Escherichia coli Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter*, *Staphylococcus areus*, and *Micrococcus luteus*. Nearly all fractions exhibited more or less antimicrobial activity against the test strains as described in earlier reports (Jolanta *et al*, 2008). The X2 (chloroform) fraction represented good antimicrobial activity against *Staphylococcus aureus* with significant MIC value. This was comparatively less than the reports of a recently published research (Maria *et al.*, 2010). This variation may be due to geographical location of plant however, certainly in both cases it is important that MIC values are too high to be categorized in susceptible ranges (Paul *et al.*, 2006). The MBC values of fraction X2 (chloroform) are 2 times the MIC as reported previously (Maria *et al.*, 2010, Anon *et al*, 2003).

Nearly all fraction of *Circium arvense* were primarily reported as active against the *Aspergillus niger* in spite of the fact that the inhibitory zones observed were not significantly wide. This report is probably the first to explore the antifungal activities of *Circium arvense*, as a comprehensive literature review give no information about the antifungal activities of this plant.

CONCLUSION

It is concluded that the *Circium arvense* having potential antibacterial and antifungal activities, irrespective of fact that the MIC values reported were high and do not prove the plant good for treatment of multiple infections. As this is used as one of the ingredients of traditional medicine in some parts of world including Pakistan, a synergistic effect is expected regarding healing of wounds if used with other ingredients. Further investigations are recommended to exploit the hidden medicinal value of plant.

REFERENCES

Anon (2000). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution. *Clin. Microbiol. Infect.*, **6**: 509-515.

Anon (2002). Traditional Medicine Strategy 2002-2005. Edited by WHO/EDM/TRM. World Health Organization, Geneva, p.74.

Anon (2003). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution. *Clin. Microbiol. Infect.*, **9**: 1-7.

Ashraf M, Ali Q and Iqbal (2006). Changes in chemical composition of fixed and essential oil of black cumin (*Nigella sativa* L.) seeds collected from plants grown at different soil nitrogen habitats. *J. Sci. F. Agri.*, **86**: 871-876.

Augustin M and Hock Y (2004). Phytotherapie bei Hauterkrankungen. Urban und Fischer. *Mu.¨ nchen.*, **4**: 1-7.

Donald WW (1994). The biology of Canada thistle (*Cirsium arvense*). *Rev. Weed Sci*., **6**: 77-101.

EUCAST Definitive Document (2000). Determination of Minimum Inhibitory Concentrations (MICs) of Antimicrobial Agents by Agar Dilution. *EUCAST Definitive Document, E*., **6**: 509-515.

Fabricant DS and Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery. *Environ. Heal. Persp*., **109**: 69-75.

Goleniowski ME, Bongiovanni GA, Bongiovanni L, Palacio CO and Cantero JJ (2006). Medicinal plants from the "Sierra de Comechingones." Argentina *J. Ethnopharmacol*., **107**: 324-341.

Hadacek F and Greger H (2000). Testing of antifungal natural products: methodologies,

comparability of results and assay choice. *Phytochem. Analys*., 11: 137-147.

Ieven M, Berghe V, Mertens DA, Vlietinck F and Lammens AE (1979). Screening of higher plants for biological activities. *Plant. Medic*., 36: 311-321.

Jolanta N, Sylwia K, and Czechowska R., (2008). Polyphenolic compounds and *in vitro* antimicrobial and antioxidant activity of aqueous extracts from leaves of some *Cirsium* species. *Nat. Pro. Res*., **22**: 1583-1588.

Konig GM, Wright AD and Franzblau SG (2000). Assessment of antimycobacterial activity of a series of mainly marine derived natural products. *Plant. Medic*., **66**: 337-342.

Leininger WC (1988). Non-chemical alternatives for managing selected plant species in the western United States. XCM-118. Fort Collins, CO. Colorado State University, Cooperative Extension. In cooperation with: U.S. Department of the Interior, Fish and Wildlife Service, 47(13038).

Maria HB, Sylwia K, Czechowska M, Renata A, Katarzyna S, Jolanta N, Jerzy P, and Valery AI (2010). Enhancement of antibacterial effects of extracts from *Cirsium* species using sodium picolinate and estimation of their toxicity. *Nat Prod Res.*, **24**: 554-561.

Morishita DW (1999). Biology and management of noxious rangeland weeds. Corvallis, OR: Oregon State University Press, 162-174. [35719].

Mukherjee PK (2002). Quality Control of Herbal Drugs, an Approach to Evaluation of Botanicals. Business Horizons, New Delhi, India, p.256.

Myers RL (2000). Fire in tropical and subtropical ecosystems. In: Brown, James K., Smith, Jane Kapler, eds. Wildland fire in ecosystems: Effects of fire on flora. Gen. Tech. Rep. RMRS-GTR-42-vol. 2. Ogden, UT: U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, pp.161-173.

Norton SA (2000). Botanical heritage of dermatology. In: Avalos. (Eds.), Dermatologic Botany, CRC Press LCC, Boca Raton.

Parendes LA and Jones JA (2000). Role of light availability and dispersal in exotic plant invasion along roads and streams in the H. J. Andrews Experimental Forest, Oregon. *Conservation Biology*, **14**(1): 64-75.

Paul CJ, Arnold L, Vlietinck VBD and Vanden ML (2006) Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept. *J Ethnopharmacol*., 106: 290–302.

Raven JA, Edwards D (2001). "Roots: evolutionary origins and biogeochemical significance". *J. Experi. Bot.*, **52**: 381-401.

Toda M, Okubo S, Hiyoshi R, Shimamura T (1989). The bactericidal activity of tea and coffee. *Leter. Appl. Microbiol*., **8**: 123-125.

Yoshikazu S, Murata H, Nakanishi T and Inatomi Y (2001). Inhibitory effect of plant extracts on production of Verotoxin by enterohemorrhagic *Escherichia coli* O157: H7. *J. Healt. Sci*., **47**: 473-477.

Zouhar K (2001). *Cirsium arvense*. *In*: Fire Effect Information System (Online). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer) Available: http://www.fs.fed.us/database/feis/