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Full Length Research Paper

Biological activities of *Rumex dentatus* L: Evaluation of methanol and hexane extracts

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Rumex dentatus **L. (Plygonaceae) extracts were evaluated for antibacterial, antifungal, cytotoxic, antitumor and allopathic potential. The leaf, stem and root extracts were prepared in methanol and hexane by simple maceration. The methanol extracts of root and shoot were found effective against all the bacterial strains tested. Zone of inhibition ranged between 9.7 to 12.1 mm. While the hexane extracts inhibited fungal growth (up to 80%) more efficiently than the methanol extracts. Concentration of different the extracts of *R. dentatus* effectively inhibited tumor induction on the potato discs produced by wild type *Agrobacterium* strains At10 and At6. The root extracts either in methanol or hexane showed LD_{50} values below 1000 ppm in brine shrimp mortality assay. The methanol extracts of leaf and stem inhibited radish seed germination (70 and 61% respectively) and root length more than the hexane extracts. The *R. dentatus* methanol extract showed presence of alkaloids, saponins, anthraquinones and tannins while flavonoids were also found in both methanol as well as hexane extract.**

Key words: Antibacterial, antifungal, antitumor, allelopathic, cytotoxic, *Rumex detatus.*

INTRODUCTION

Worldwide, about 4 billion people today rely on plants as sources of drugs even in developed countries. Presently, at least 25% of standard drugs prescribed by a physician originate from folk medicines (Farnsworth, 1988). Therefore, it is a need to investigate traditional medicine with a view to identify and exploit safe and effective remedies for ailments of both microbial and non-microbial origin.

Rumex dentatus L. (Plygonaceae) is commonly known as dentate dock, Indian Dock and Toothed Dock. Traditionally *Rumex* plants are used as bactericidal (Yildirim et al., 2001), anti-inflammatory (Suleyman et al., 1999), antitumor, astringent and anti dermatitis (Litvinenko and MuzychKina, 2003) diuretic, cholagogue, tonic and laxative agents (Demirezer, 1993). Leaves, stems and roots of *Rumex abyssinicus* L., *Rumex bequaertii* L. *and Rumex usambarensis* L. are used to treat pneumonia, cough, abscesses, stomach-ache and smallpox (Midiwo et al., 2002).

There are some reports in literature about evaluation of a few species of *Rumex* for various medicinal potential. For example, the methanol extract of roots of *Rumex nepa-lensis* L. has shown significant antibacterial activity (Ghosh et al., 2001). In another study, Anthraquinones were identified as the mulluscicidal compound in *Rumex palmatum* L. and *R. dentatus* (Liu et al., 1997). The methanol extracts of fruit of *Rumex cyprius* is also reported to have antiviral activity (El-Mekkawy et al., 1995). Furthermore, Zhu and coworkers (2006) isolated ten compounds from *R. dentatus* while many important phytochemicals including emodin, aloe emodin, chrysophanol (Elkey et al., 1964) physocin (Fairbairn and El-Muhtadi, 1972) chrysophanol, parietin and nepodine (Choi et al., 2004) have previously been isolated from different species of *Rumex* genus.

Plants contain thousands of biologically active molecules. For their investigation, it is important to have the necessary tools. These include suitable biological assays

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and chemical screening methods. The search for new antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fetal opportunistic infections (Kivack et al., 2001). The discovery and development of new antimicrobial agents is a continuous process. Remarkable diversity of chemicals present in biological samples has tremendous potential in search of new antimicrobial agents. Almost all of the antifungal agents, that are currently in use are relatively expensive and have toxic side effects (Morens et al., 2004).

Bioassay methods used in assessing the antitumor activity of plant extract have varied over years. These methods have yielded important discoveries including vincristine, vinblastin the podophyllotoxin derivatives, 10-hydroxy-campothecin and Taxol. Initial screening for antitumor potential by using *Agrobacterium tumefaciens* (crown gall tumor formation on potato discs) could be used as a fairly rapid, inexpensive and reliable prescreen for antitumor agent (Galsky et al., 1980) along with cytotoxicity assay that present good correlation between brine shrimp toxicity and human nasopharyngeal carcinoma (McLaughlin and Rogers, 1998).

Radish (*Raphanus sahvus* L.) seeds have been used in general toxicity studies because of their sensitivity to phytotoxic compounds (De-Feo et al., 2003) and is a standard assay in allelopathic studies (Patterson et al., 1986). This also provides basic idea to use or isolate such compounds comprising herbicidal potential.

Present study was aimed to screen crude *R. dentatus* extracts for antibacterial, antifungal, antitumor, cytotoxic and allelopathic potential along with their phytochemical evaluation.

MATERIALS AND METHODS

Collection of the plant material and extraction procedure

The plant material (*R. dentatus*) was collected at its flowering stage from District Hafizabad, Punjab, Pakistan during the month of February. The whole plant material was rinsed with distilled water and kept under shade for drying. Extraction was carried out by simple maceration.

The leaves shoot and roots were separated and weighed. Each part was divided into two halves. One half of the total weight of 900 g of each part was blended into 2 L of *n*-hexane and the other in methanol separately. The mixtures were kept in closed container for 14 days at room temperature (25 ± 2°C) with occasional shaking. After 14 days the mixtures were filtered and excessive solvent was evaporated by rotary evaporator.

The extracts obtained were labeled as HL 25 g (hexane extract of leaves), HR 20 g (hexane extract of root) and HS 22 g (hexane extract of shoot). Similarly ML, MR and MS (145, 152 and 130 g, respectively), were methanol extracts.

Antibacterial assay

Antibacterial assay was performed according to standard agar well diffusion method. Nine bacterial strains (*Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus, Eschericia coli, S. Setubal, Pseudomonas picketii, Bordetella bronchiseptica* and two *Agrobacterium tumefaciens* strains) were tested. A 24 h old bacterial culture was inoculated in pre-autoclaved nutrient agar medium and poured in petri plates. After solidification, wells were made by 8 mm cork borer and each well was labeled. A volume of 100 µl of extract (20 mg/ml in DMSO), Cefotaxime (2 mg/ml in DMSO) as positive control and pure DMSO as negative control were poured in respective wells. Plates containing *Agrobacterium* strains were incubated at 28°C and of others bacterial strains at 37°C. Zone of inhibition was recorded after 24 h of incubation.

Antifungal assay

The agar tube dilution method was performed to determine antifungal activity of plant extract (Choudhary et al., 1995). Media for fungi was prepared by mixing 32.5 g savored dextrose agar (Mark) in 500 ml distilled water. It was then steamed to be dissolved and 5 ml was dispensed into screw cap tubes. Tubes were labeled and autoclaved at 121°C for 20 min.

Tubes were allowed to cool and just before solidification, 100 µl of plant extract (20 mg/ml in DMSO) and 83 µl of terbinafine (12 mg/ml in DMSO) as positive control was added in tubes to get concentration of 400 and 200 µg/ml respectively. Pure DMSO (100 µl/tube) was used as negative control. Tubes were allowed to solidify at slanting position at room temperature. Each tube was inoculated with a 4 mm diameter piece of inoculum from a seven days old culture. The tubes were incubated at 27°C for 7 days. Growth was determined by measuring linear growth (mm). Test was performed in triplicate and growth inhibition was calculated with reference to negative control with the help of following formula: % inhibition of fungal growth = 100 - (Linear growth in test / Linear growth in control) x 100.

Cytotoxicity (brine shrimp) assay

The plant extracts (20 mg) were dissolved in 2 ml of respective solvent (methanol/ hexane). From this stock solution 5, 50 and 500 µl was poured separately in, pre marked at 5 ml; 20 ml vials (3vials/concentration) to attain final concentration 10, 100, 1000 ppm respectively. The vials were kept open over night with continuous air flow to evaporate the solvent.

Artificial sea water (3 ml) was poured in each vial and 10 matured brine shrimp larvae were added with the help of pasture pipette. Final volume in each vial was increased at marked level by adding sea water. The vials were kept under illumination and after 24 h, survived nauplii were counted macroscopically. ED_{50} value was calculated by probit analysis in a finny computer program.

Antitumor potato disc assay

Antitumor assay was performed according to procedure described by McLaughlin and Rogers (1998). In brief, *A. tumefaciens* strains At 6 and At 10 were cultured in LB medium for 48 h. Inoculum containing three concentrations of the extracts (10, 100 and 1000 ppm), *Agrobacterium* culture and DMSO were made.

For assay, red skinned potatoes were purchased from local market. Under aseptic conditions these potatoes were surface sterilized with 1% $HgCl_2$ solution for 4 - 5 min and rinsed thrice with distilled autoclaved water. Potato discs of height 2 mm and diameter 8 mm were made with the help of cork borer. Ten discs were placed on petri plates containing autoclaved agar medium (1.5%). On the top surface of each potato disc 50 µl inoculum was poured. The plates were sealed with parafilm and incubated at 28°C in dark. After 21 days, potato discs were stained with Lugol's solution (10% KI + 5% I2) and tumors were counted under dissecting microscope. Test was performed in triplicate and data was statistically analyzed

Table 1. Antibacterial activities of crude extracts of *Rumex dentatus* against some bacterial strains.

Sample	*S. aureus*	*B. subtilis*	*M.luteus*	*E. coli*	*S. setubal*	*P. picketii*	*B. bronchisptica*	*A. tumefaciens*	
								At 6	At 77
Cefotaxime	31.3 ± 0.57	27.1 ± 0.28	32.0 ± 0.11	40.0 ± 0.05	31.3 ± 0.57	26.1 ± 0.17	25.0 ± 0.23	25.3 ± 0.05	28.5 ± 0.16
MR	10.2 ± 0.05	11.1 ± 0.11	10.3 ± 0.05	10.4 ± 0.05	11.0 ± 0.05	9.5 ± 0.05	12.1 ± 0.05	10.3 ± 0.05	10.5 ± 0.05
HR	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
MS	10.0 ± 0.17	9.7 ± 0.05	10.0 ± 0.00	10.3 ±0.17	10.4 ± 0.05	10.3 ± 0.03	10.4 ± 0.05	8.5 ± 0.57	8.6 ± 0.5
HS	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
ML	Nil	Nil	Nil	Nil	Nil	10.2 ± 0.05	Nil	Nil	Nil
HL	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
DMSO	-	-	-	-	-	-	-	-	-

by ANOVA.

Phytotoxicity (radish seed) assay

The method of radish seed bioassay was followed as reported by Arzu and Camper (2002). In brief, 50 mg of each extract was dissolved in 5 ml of respective solvent (methanol and hexane) to get 10,000 µg/ml concentrations. The solution was poured in petri plate containing sterilized Whatman #1 filter paper and left in laminar flow till evaporation of the solvent. In each petri plate 5 ml of distilled autoclaved water was poured. Methanol, hexane and water were used as control.

Twenty five radish seeds sterilized with 0.1% mercuric chloride solution were placed in each plate. Petri plates were incubated in dim light at 25°C. Number of seeds germinated and root length was measured at 5th days. The test was performed in triplicate and data were analyzed by ANOVA.

Phytochemical analysis

Phytochemical tests for alkaloids, saponins, anthraquinones, coumarins, flavonoids and tannins were performed for each extract according to standard protocols.

RESULTS AND DISCUSSION

Antibacterial activity

The result shows that methanol extracts of roots and shoots had antibacterial potential against all strains (Table 1). Maximum zone of inhibition (12.1 mm) was observed against *Bordetella* by methanol extract of roots followed by activity against *Salmonella* and *Bacillus* (zone of inhibition 11.00 and 11.1 respectively) by the same extract. Methanol extract of the shoot of *R. dentatus* had on average 10.0 mm zone of inhibition against all bacterial strains. The leaves extract did not show any activity against bacterial strains except *Pseudomonas*. Hexane extract of leaves, shoots and roots did not show any activity against any bacterial strain tested. Methanol proved better solvent for extraction of antibacterial constituent as compared to hexane which allows polarity solvent. Vlachos et al. (1996) also concluded that methanol was the most effective solvent for the extraction of antibacterial compounds from the selected seaweed. Methanol extract of roots of *R. nepalensis* and aerial parts of *Rumex crispus* has been determined for antibacterial potential (Ulukanli et al., 2005; Ghosh et al., 2001).

While bactericidal activity of methanol extract of roots was found. Mean zone of inhibition by methanol extract of root against At 6 and At 10 were 10.3 mm and 10.5 mm respectively. Very small zone of inhibition zone was observed by methanol extract of shoots. Other plant extracts were not found effective against both *Agrobacterium* strains tested. Inayatullah et al. (2007) in reported no affect of different plant extracts on *A. tumefaciens* strains while working on different plant species.

Phytochemical analysis exhibited that methanol extracts contain anthraquinones that may behave as antibacterial agent. Anthraquinones extracted from different species of *Aloe* also exhibit antibacterial activity against *B. subtilis* (Levin et al., 1988).

Antifungal activity

The results show that hexane extracts inhibited

Table 2. Effect of crude extract of *Rumex dentatus* on percentage inhibition of fungi.

Sample	*Fusarium solani*	*Aspergillus flavas*	*Aspergillus niger*	*Mueor species*	*Alternaria alterata*	*Aspergillus fumigatus*	*Fusarium moniliformes*
MR	53	10	56	20	22	42	75
HR	78	61	75	80	75	40	25
ML	77	09	68	11	05	17	08
HL	82	80	75	72	50	53	65
MS	10	07	27	39	27	04	10
HS	20	45	75	73	68	10	80
Stand. Drug	100	100	100	100	100	100	100
DMSO	-	-	-	-	-	-	-

Table 3. Percentage mortality of brine shrimp and respective LD_{50} values.

Extracts	Percentage death after 24 h			LD_{50}
	10 ppm	100 ppm	1000 ppm	
MR	13.33	16.66	56.66	867.80
ML	20.00	26.66	50.00	1371.80
MS	16.60	20.00	50.00	1552.10
HR	13.33	20.00	66.66	437.40
HL	13.33	23.33	30.00	53970.99
HS	23.33	30.00	43.33	4753.81

fungal growth more efficiently as compared to methanol extract (Table 2). Maximum inhibition was observed by roots and leaves hexane extract where percentage inhibition ranged from 60-82 against all fungal strains except *Aspergillus fumigatus*. Methanol extracts of leaves, shoots and roots did not show any prominent inhibition. Maxi-mum inhibition that is, 75 and 77% was observed against *Fusaium moniliformes* and *Fusaium solani,* respectively by methanol extracts of roots and leaves. Phytochemical analysis of hexane extracts showed presence of flavor-noids as major constituents, which might be responsible for antifungal activity (Atindehou et al., 2002). Previously, fungitoxic surface flavonoids (Isoflavonoids) have been characterized on leaf and root surface and are reported to be extracted with non polar or low polar solvents (Harbone, 1999). Chromone, a secondary metabolite pre-sent in crude *n*-hexane extract of shoot of *Ageratum conyzoides* completely inhibited the growth of *Rhizoc-tonia solani* and *Sclerotium rolfsii.* The presence of chromones in *R. dentatus* has been reported (Zhu et al., 2006) which might have antifungal effect.

Methanol extracts of shoot showed minor activity while ML and MR showed significant activity against *F. solani, Aspergillus niger* and *F. moniliformes.* Methanol extract of roots of *Rumex crispus* has been reported to be active against *Erysiphe graminis* (Kim et al., 2004).

Cytotoxicity and antitumor assay

Brine shrimp assay is suggested to be a convenient probe for the pharmacological activities in plant extract (Mayer et al., 1982). Historically, different scientist worked out to access the cytotoxic activity of different plant extracts by using different testing organisms. Re-sults pertaining to cytotoxicity are shown in Table 3. None of the plant extracts were found highly effective ($P > 0.05$) however, at higher concentration, plant extracts was effective at probability level 0.05. LD_{50} values describe that the hexane extract of root was most effective with LD_{50} 437.40. Mortality of brine shrimp by this extract was maximum (66.60%) at concentration of 1000 ppm. The methanol extract of root was also effective with mortality value of 56.60%. Other extracts did not show prominent results, where the mortality of brine shrimp was near by 50% or below it even at higher concentration which resulted in higher vales of LD_{50} for these extracts. A pre-vious report shows that aqueous extract of roots of *R. dentatus* has moderate mulluscicidal activity against snail's larvae (Liu et al., 1997) while minor effect of aqueous extract of *R. crispus* against brine shrimp (*Arte-mia salina*) has also been described (Krishnaraju et al., 2006).

A. tumefaciens was used as the tumor causing agent in potato disc assay because of its unique capacity for

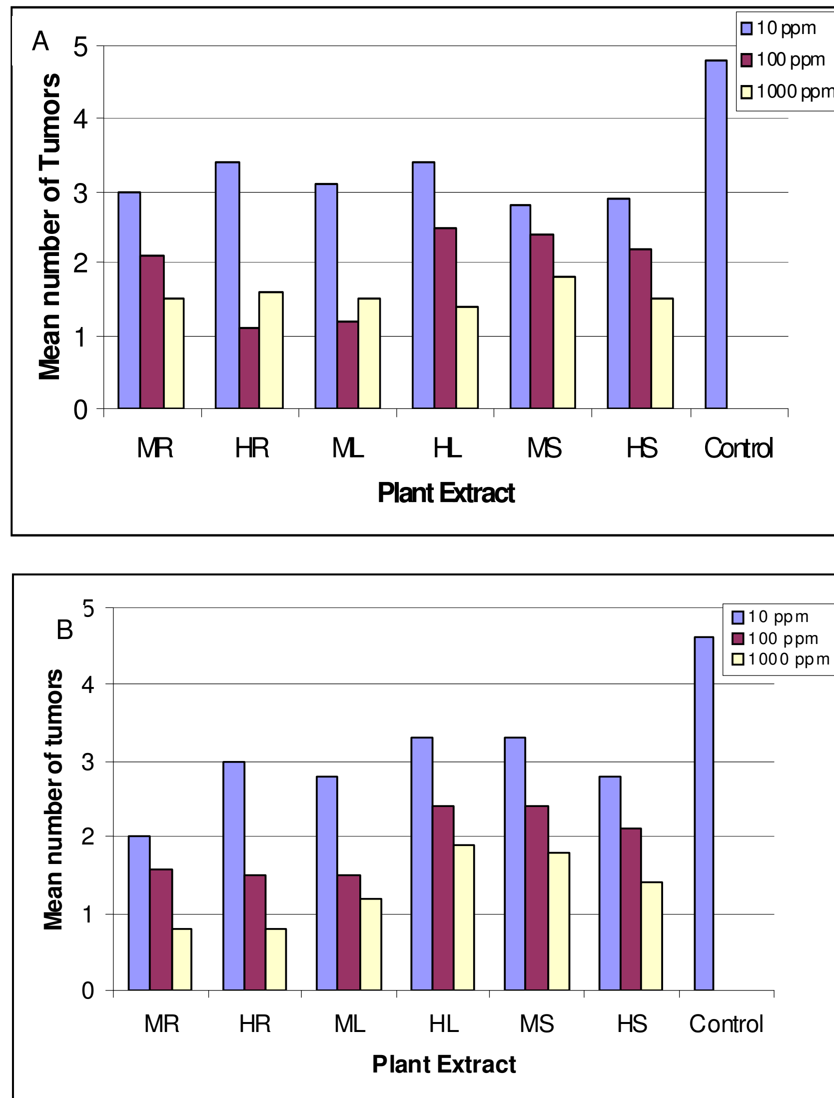

Figure 1. Effect of *Rumex dentatus* extracts on tumor induction (A) by At 6, (B) At 10.

trans- kingdom sex that is, transfer of genetic material between prokaryotic and eukaryotic cells (Stachel and Zambryski, 1998). Effect of the both methanol and hexane extracts on tumor induction were variable and non significant. The only factor which affected tumor induction significantly was concentration of the samples. Tumor inhibition results were non-significant at probability level $P < 0.05$ in the case of plant. Figure 1 (a and b) shows mean number of tumors (out of 10) either produced by At6 or At10. Average number of tumors produced by At6 and At10 were 4.8 and 4.6, respectively.

Although there are reports on antitumor activity of *Rumex hymenosepalus* and *R. dentatus* (Chen et al., 2003). It may be assumed that environmental and physiological factors may be different in above given case that resulted in different activities. Environmental and physiological factors have critical role in activity of natural products in plants (Hansen et al., 2005).

Radish seed (phytotoxicity) assay

Radish seed germination assay is important to define allelopathic potential of extracts. Seed germination and root length results were significant at 5% probability level. Maximum seed germination inhibition that is, 70 and 61% after 5th day of incubation was observed by methanol extract of leaves and shoots, respectively as shown in Figure 2. Methanol extract of root inhibited the germination by 47% while low or no activity was observed by hexane extracts of leaves, shoots and roots.

Results were also significant ($p < 0.05$) in case of

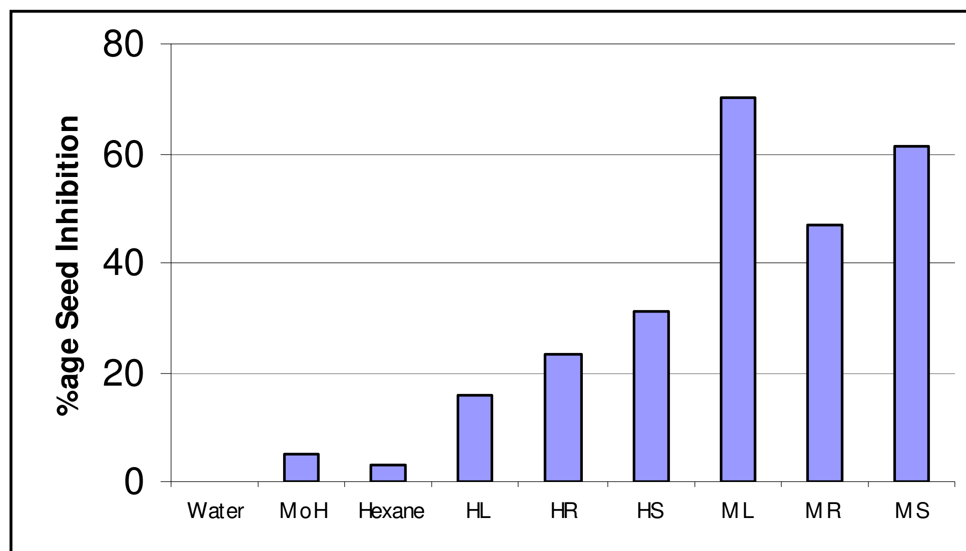

Figure 2. Percentage of radish seed inhibition by *Rumex dentatus* extract.

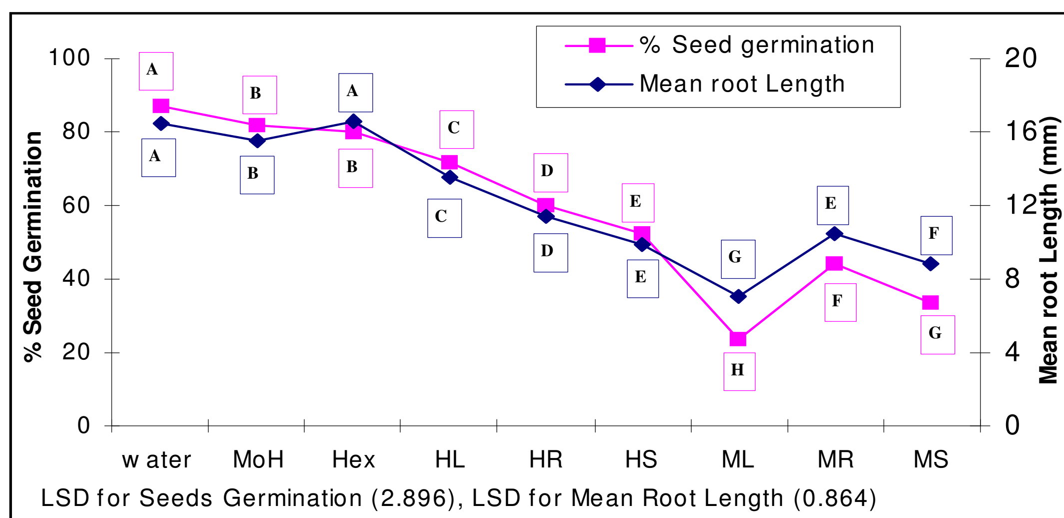

Figure 3. Effect of *Rumex dentatus* extract on radish seed germination and root length.

radish seedling root length (Figure 3). Minimum root length (7.07 mm) was observed when radish seeds were grown in the presence of methanol extract of leaves of *R. dentatus*. These results are in agreement to a previous report (Carral et al., 1987) that extracts of leaves of *Rumex obtufisifolius* were most toxic for germination and root growth of meadow species. An inhibitory effect of *R. crispus* extracts on *Amaranthas retroflexus,* grain *sorghum* and field corn has been proven (Einhellig and Rasmussen, 1973). After chromatographic analysis they concluded that phenolics were responsible to inhibit seed germination and root length. While, Inderjit (1996) reported that phenolics are major class that have allelopathic potential. Phytochemical analysis showed presence of many classes of polyphenols including anthraquinones that might be responsible for allelopathic effect.

Phytochemical analysis

Phytochemical analysis of hexane and methanol extract of leaves, shoots and roots showed that flavonoides were present in all hexane and methanol extracts of *R. dentatus.* While alkaloids and saponins were only present in methanol extracts of leaves, shoots and roots. Anthraquinones were present only in methanol extracts in ranking order roots > shoots > leaves. Different types of tannins depending upon test color were present in

methanol extracts of roots, leaves and shoots.

Conclusions

A number of reports describe biological activities of plant extracts to be used for further phytochemical investigations (Faizi et al., 2008; Yasmin et al., 2008; Inayatullah et al., 2007). This report also shows that *R. detatus* extract has potential to be used for isolation of antimicrobial, allelochemicals and other chemotherapeutic agents. Such discoveries are not only helpful to be used against animal/human disease driving forces but can also be used against plants intrusions such as crown gall disease.

REFERENCES

Arzu UT, Camper ND (2002). Biological activity of common mullein, a medicinal plant. J. Ethnopharm. 82: 117-125.

Atindehou KK, Queiroz EF, Terreaux CTD, Hostettman K (2002). Three new prenylated isoflavonoids from the root, bark of *Erythrina vogelii.* Planta Med. 68: 181-182.

Carral E, Reigosa MJ, Carballeira A (1987). *Rumex obtusifolius* L: Release of allelochemical agents and their influence on small-scale spatial distribution of meadow species. J. Chem. Ecol. 14(9): 1763-1773.

Chen X, Ung CY, Chen Y (2003). Can an *in Silico* drug-target search method be used to probe potential mechanisms of medicinal plant ingredients? Nat. Prod. Rep. 20: 432-444.

Choi GJ, Lee SW, Jang KS, Kim JS, Cho KY, Kim JC (2004). Effect of chrysophanol, parietin and nepodin of *Rumex crispus* on barley and cucumber powdery mildews. Crop Prot. 23(12): 1215-1221.

Choudhary MI, Shahwar D, Parveen Z, Jabbar A, Ali I, Rahman A (1995). Antifungal steroidal lactones from *Withania coagulance.* Phytochemistry, 40(4): 1243-1246.

De-Feo V, Martino L, Quaranta E, Pizza C (2003). Isolation of phytotoxic compounds from tree-of-heaven (*Alianthus altissima* Swingle). J. Agric. Food Chem. 51(5): 1177-1180.

Demirezer LO (1993). Comparison of two *Rumex* Species with a spectrophotometric method and chromatographic identification with regard to anthraquinone derivatives. Planta Med. 59: p. 630.

Einhellig FA, Rasmussen JA (1973). Allelopathic Effects of *Rumex crispus* on *Amaranthus retroflexus,* Grain *Sorghum* and Field corn. J. Am. Midland Natarutlist 90(1): 79-86.

Elkey MA, Darwish S, Moustafa MA (1964). Investigation of the volatile oil content of certain Eucalyptus species cultivated in Egypt. Bull. Fac. Pharm. 3(1): 97-108.

El-Mekkawy S, Meselhy MR, Kusumoto IT, Kadota S, Hattori M, Namba T (1995). Inhibitory effects of Egyptian folk medicines on human immunodeficiency virus (HIV) reverse transcriptase. Chem. Pharm. Bull. 43(4): 641-648.

Fairbairn JW, El-Muhtadi FJ (1972). Chemotaxonomy of anthraquinones in *Rumex*. Phytochemistry, 11: 263-268.

Faizi S, Siddiqi H, Bano S, Naz A, Lubna MK, Nasim S, Riaz T, Kamal S, Ahmad A, Khan SA (2008). Antibacterial and antifungal activities of different parts of tagetes patula: Preparation of patuletin derivatives. Pharm. Biol. 46: 309-320

Farnsworth NR (1988). In: Biodiversity, Willson, E. O. (eds.) National Academy Press, Washington D. C. pp. 83-97.

Galsky AB, Wilsey JP, Powell RG (1980). Crown-gall tumor disc bioassay: A possible aid in the detection of compounds with antitumor activity. Plant Physiol. 65: 184-185.

Ghosh L, Gayen JR, Sinha S, Pal S, Pal M, Saha BP (2001). Antibacterial efficacy of *Rumex nepalensis* Spreng roots. J. Phytother. Res. 17(5): 558-559.

Hansen EH, Schafer T, Molin S, Gram L (2005). Effect of environmental and physiological factors on the antibacterial activity of *Curvularia haloper* oxidase system against *Escherichia coli.* J. Appl. Microbiol. 98(3): 581-588.

Harbone JB (1999). Classes and Functions of Secondary Products from Plants in; Brown DE and Walton NJ (eds). Chemical from plants perspective on plants secondary products. Imperial college Press (Lond.) p. 18

Inayatullah S, Irum R, Ateeq-ur-Rehman, Chaudhary MF, Mirza B. (2007). Biological evaluation of some selected plant species of Pakistan. Pharm. Biol. 45: 397-403.

Inderjit (1996). Plant phenolics in allelopathy. Bot. Rev. 62: 186-202.

Kim JC, Choi GJ, Lee SW, Kim JS, Chung KY, Cho KY (2004). Screening extracts of *Achyranthes japonica* and *Rumex crispus* for activity against various plant pathogenic fungi and control of powdery mildew. Pest Manage. Sci. 60(8): 803-804.

Kivack B, Mert T, Tansel H (2001). Antimicrobial and Cytotoxic activites of *Ceratonia siiqual* L. extracts. Turk. J. Biol. 26: 197-200.

Krishnaraju AV, Tayi VN, Rao SD, Vanisree M, Tsay HS, Subbaraju GV (2006). Biological Screening of Medicinal Plants Collected from Eastern Ghats of India Using *Artemia salina* (Brine Shrimp Test). Int. J. Appl. Sci. Eng. 4(2): 115-125.

Levin H, Hazenfrantz R, Friedman J, Perl M (1988). Partial purification and some properties of the antibacterial compounds from *Aloe vera.* Phytother. Res. 1: 1-3.

Litvinenko YA, MuzychKina RA (2003). Phytochemical investigation of biologically active substances in certain Kazakhstan *Rume*x species. Chem. Nat. Comp. 39(5): 368-370.

Liu SY, Sporer F, Wink M, Jourdane J, Henning R, Li YL, Ruppel A (1997). Anthraquinones in *Rheum palmatum* and *Rumex dentatus* (Polygonaceae) and phorhol esters in *Jatropha curcas* (Euphorbiaceae) with molluscicidal activity against the schistosome vector snails *Oncomelania*, *Biomphalaria* and *Bulinus*. Trop. Med. Int. Health, 2(2): 179-188.

Mayer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL, Rogers LL (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 45: 31-54.

McLaughlin R (1998). The use of biological assays to evaluate botanicals. Drug Inform. J. 32: 513-524.

Midiwo JO, Yenesew A, Juma BF, Derese S, Ayoo JA, Aluoch AO. Guchu S (2002). Bioactive compounds from some Kenyan Ethanomedicinal plants: *Myrisnaceae, Polygonaceae* and *Psiadia punctulata*. Phytochem. Rev. 1: 311-323.

Morens DM, Folkers GK, Fauci AS (2004). The challenge of emerging and re-emerging infections diseases nature. Nature, 430: 242-249.

Patterson TD, Ann ER, David AM, Robert DC, Elizabeth PF (1986). Effects of Temperature and Photoperiod on Texas Panicum (*Panicum texanum*) and Wild Proso Millet (*Panicum miliaceum*). Weed Sci. 34(6): 876-882.

Stachel SE, Zambryski PC (1989). Generic trans- kingdom sex. Nature, 340: 190-191.

Suleyman H, Demirezer LO, Kuruuzum ZN, Lu B, Gocer F, Ozbakir G, Gepdiremen A (1999). Antiinflamanatory effect of aqueous extract from *Rumex patientia* L. roots. J. Ethnopharm. 65(2): 141-148.

Ulukanli Z, Ulukanli S, Ozbay H, Ilcim A, Tuzcu M (2005). Antimicrobial Activities of Some Plants from the Eastern Anatolia Region of Turkey. Pharm. Biol. 43(4): 334-339.

Vlachos V, Critchely AT, Von HA (1996). Establishment of a protocol for testing antimicrobial activity in Southern African macroalgae. Microbios. 88: 115-123.

Yasmin S, Kashmiri MA, Ahmad I, Adnan A, Ahmad M (2008). Biological activity of extracts in relationship to structure of pure isolates of *Abutilon indicum*. Pharm. Biol. 46: 673-676

Yildirim A, Mavi A, Kara AA (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem. 49(8): 4083-4089.

Zhu JJ, Zhang CF, Zhang M, Wang ZT (2006). Anthraquinones and chromones from *Rumex dentatus*. Biochem. Syst. Ecol. 33(10): 753-756.