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Pharmacological and Biological Investigations of *Chenopodium murale* Linn.

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Abstract: Crude methanolic extract of *Chenopodium murale* Linn. whole plant and its various fractions obtained in different solvent systems, was screened for different pharmacological and biological activities such as antifungal, antibacterial, insecticidal, phytotoxic and brine-shrimp cytotoxic activities. The extract and all the fractions exhibited well to excellent activities against different pathogens in antifungal bioassay and mild to moderate activities against different pathogens in antibacterial bioassay. While they did not display any significant insecticidal and brine-shrimp cytotoxic activities. Also the crude extract and its various fractions (except aqueous) showed remarkable phytotoxic activities against *Lemna acquinoctialis* welv. at highest concentration (1000 μg ml^{-1}). However, they displayed weak phytotoxic activities at lower concentrations (100 μg ml^{-1}), while at further lower concentration (10 μg ml^{-1}) some of them rather promoted the growth of Lemna *acquinoctialis* welv. The aqueous fraction promoted the growth of *Lemna acquinoctialis* welv. at all tested concentrations.

Key words: *Chenopodium murale* Linn., antifungal bioassay, phytotoxic activities, *Lemna acquinoctialis* welv.

INTRODUCTION

Chenopodium murale Linn. belongs to the family Chenopodiaceae also called as goose foot family that contains about 102 genera and 1400 species of annual and perennial herbs and shrubs scattered throughout the world (Marie, 1965). The genus *Chenopodium* consists of 200 species (Boulos, 1983).

Chenopodium murale is an erect annual plant, up to 60 cm in height. It is slightly mealy and its leaves are 2-8 x 1-6 cm broad, ovate, angular sides, lobed, sharply toothed, base wedge shaped and stalked long or short. Flowers are in slender spikes, forming loose dense axillary clusters. Seeds are sharply keeled, dotted and horizontal (Bamber, 1916 and Davis, 1967).

Plants belonging to the genus *Chenopodium* are reported to have wide applications in folk medicines; as an anthelmintic, stomachic, antispasmodic, diaphoretic, emmenagogue, for the pain of amenorrhea, as an abortifacient and for the relief of asthma, catarrh and migraine (Watt and Breyer, 1962 and Vasishita, 1989). In Mexico, *Chenopodium* is used for medicinal purpose to treat different illnesses, conditions or discomfort, e.g., sterility, digestive problems, anxiety, depression, hair loss and cough etc (Vega and Iskander, 1997). Some flavonoids isolated from *C. murale* showed antihypertensive activity (Ahmad and Elmazar, 1997). Various *Chenopodium* species have been reported to have anthelmintic properties (Lozoya J. and M. Lozoya, 1982).

From the phytochemical point of view, the chenopods were reported to contain essential oils (Rustenbekova *et al*., 1974; De-Paschual *et al*., 1983), a wide variety of flavonoids (Bahrman *et al*., 1985; Ahmad *et al*., 2000; El-Sayed *et al*., 1999), sterols and steroidal oestrogens like substances (Van-Rompuy and Zeevaart, 1979; Bathory *et al*., 1982), alkaloids and coumarins (Rizk, 1986a; Rizk, 1986b).

Aims and objectives of the study: The aims and objectives of this study was:

- To provide scientific basis for the use of this plant in the treatment of various diseases.
- To pave way for discovering the active principals of the plant for medicinal purposes.
- To identify scientifically proven indigenous herbal medicinal source for use by patients at affordable prices.

For this purpose crude extract of *Chenopodium murale* Linn. in methanol and various fractions thereof in different organic solvents like *n*- hexane, chloroform, ethylacetate and *n*- butanol was screened for various pharmacological and biological activities such as antifungal, antibacterial, insecticidal, brine-shrimp cytotoxic and phytotoxic bioassays.

MATERIALS AND METHODS

Plant material: The plant was collected from Matta, District Swat, NWFP, Pakistan in the month of July 2001. The plant was identified by Mehboob-ur-Rehman, plant taxonomist Government College Matta, District Swat, NWFP, Pakistan and verified by Dr.Abdur-Rashid, Chairman Department of Botany, University of Peshawar, NWFP, Pakistan, where voucher specimens were deposited in their Herbarium.

Extraction and fractionation: The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. The powdered plant material (10 Kg) was soaked in methanol (3 x 10 L), with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator. A dark greenish crude extract (253 g) was obtained.

The crude methanolic extract (253 g) was suspended in distilled water (500 ml) and partitioned with *n*-hexane (3 x 500 ml), chloroform (3 x 500 ml), ethyl acetate (3 x 500 ml) and *n*-butanol (3 x 500 ml) to yield the *n*-hexane (24 g), chloroform (61 g), ethyl acetate (30 g), *n*-butanol (35 g) and aqueous (73 g) fractions, respectively.

Screening of extract and its various fractions for different pharmacological and biological activities: The crude methanolic extract and various fractions thereof including chloroform, ethyl acetate, *n*-butanol and aqueous fractions were screened for different pharmacological and biological activities such as antifungal, antibacterial, insecticidal, brine-shrimp cytotoxic and phytotoxic activities.

Antifungal activities: Antifungal activity of the crude extract and various fractions were evaluated by agar tube dilution method (Atta-ur-Rehman *et al*., 1991). The samples in the concentration of 24 mg ml^{-1} were dissolved in sterile dimethyl sulfoxide (DMSO), which served as stock solution. Sabouraud dextrose agar (SDA) (4ml) was dispensed into screw cap tubes, which were autoclaved at 121 °C for 15 min. and then cooled to 50 °C. The non-solidified SDA media was poisoned with stock solution (66.6 µl), giving the final concentration of 400 µg of the extract/ml of SDA. Each tube was inoculated with a piece (4 mm diameter) of inoculum removed from a seven days old culture of fungi. For non-mycelial growth, an agar surface streak was employed. Inhibition of fungal growth was observed after 7 days of incubation at 28±1 °C. A control experiment with test substance (medium supplemented with appropriate amount of DMSO) was carried out for verification of the fungal growth.

Antibacterial activities: The crude extract and its fractions were screened against various human pathogens including, *Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Shigella flexenari, Staphylococcus aurous, Pseudomonas aeruginosa* and *Salmonella typhi,* by agar well diffusion method (Atta-ur-Rehman *et al*., 1991).

In this method 1 loop full of 24 h old culture containing approximately 10^4-10^6 CFU was spread on the surface of Mueller-Hinton Agar plates. Wells were dug in the medium with the help of a sterile metallic cork borer. Stock solutions of the test samples in the concentration of 1mg ml^{-1} were prepared in dimethyl sulfoxide (DMSO) and 100 µl and 200 µl of each dilution was added in their respective wells. Control well received only 100 $\mu g\ \mu l^{-1}$ and 200 $\mu g\ \mu l^{-1}$ of DMSO. Imipinem was used as the standard drug.

Insecticidal activities: *Tribolium castaneum*, *Rhyzopertha dominica* and *Callosbruchus analis* were used to determine the insecticidal activity of the crude extract and its various fractions (Naqvi and Parveen, 1991).

Phytotoxic activities: Phytotoxic activity of the crude extract and various fractions were carried out against the *Lemna acquinoctialis* welv. (McLaughlin *et al*., 1991). The medium was prepared by mixing various constituents in distilled water (100 ml) and the pH was adjusted (5.5 - 6.5) by adding KOH solution. The medium was then autoclaved at 121°C for 15 min. The samples (30.0 mg) dissolved in ethanol (1.5 ml) served as stock solution. Nine sterilized flasks, three for each concentration, were inoculated with 1000 µl, 100 µl and 10 µl of the stock solution to give the final concentration of 1000, 100 and 10 µg ml^{-1} respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each flask, medium (20 ml) and plants (10), each containing a rosette of three fronds, of *Lemna acquinoctialis* welv., were added. One other flask supplemented with solvent and reference growth inhibitor (Paraquate), served as negative control. All flasks were plugged with cotton and kept in the growth cabinet for seven days. The number of fronds per flask were counted and recorded on day seven.

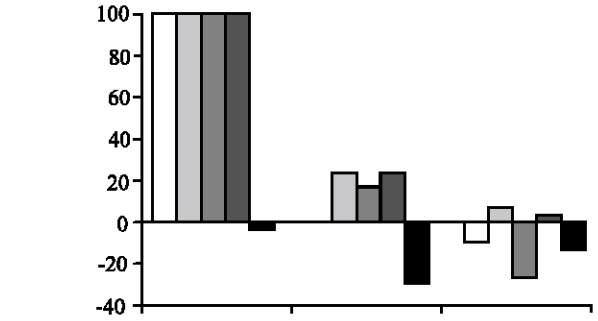
Brine-shrimp cytotoxicity: *Artemia salina* (brine-shrimp eggs) was used to determine the cytotoxic activity of the crude extract and various fractions thereof (Meyer *et al*., 1982).

RESULTS AND DISCUSSION

The shade dried plant material of whole plant of *Chenopodium murale* Linn. was macerated with methanol. The crude methanolic extract was suspended in water and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol to yield the *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions, respectively.

Antifungal activity of the crude extract and its fractions of *Chenopodium murale* were tested against *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani and Candida glaberata*. Growth in the medium containing the extracts was determined by measuring the linear growth in mm and the % growth inhibition was calculated with reference to the negative control. The results (Table 1) indicate that the crude extract exhibited a good antifungal activity (70%) against *Trichophyton longifusus* while it displayed a weak inhibition (30%) against *Microsporum canis*. The crude extract was devoid of any antifungal inhibition against the rest of tested fungi. Table 1 also shows that $CHCl_3$ fraction showed good antifungal activities against *Trichophyton longifusus* (75%), *Microsporum canis* (65%) and *Candida glaberata* (57%) while it devoid of any activity against the rest of pathogens. The EtOAc fraction displayed an overall moderate antifungal activity with an excellent activity against *Trichophyton longifusus* (80%). However, this fraction did not show any activity against *Aspergilus flavus*. The *n*-BuOH fraction showed excellent activity against *Trichophyton longifusus* (90%) while having moderate antifungal activity against *Candida albicans* (55%) and *Microsporum canis* (45%), while a weak activity against *Fusarium solani* (10%) with no activity against *Aspergilus flavus* and *Candida glaberata*. The aqueous fraction of the extract of *C. murale* displayed good activity against *Trichophyton longifusus* (65%). However, the aqueous fraction did not show any activity against the rest of fungi. Table 1 shows that all the fractions have well to excellent (65–90%) antifungal activity against the *Trichophyton longifusus*. The same table also shows that none of fractions have displayed any activity against *Aspergilus flavus*.

The antibacterial activities of the crude extract as well the fractions thereof *of C. murale* were tested against the bacteria *Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi*. The results are given in Table 2, which shows that the crude extract displayed moderate inhibitory activities against *E. coli* (50.00%), *B. subtilis* (58.06%) and *P. aeruginosa* (64.00%), while it was devoid of any antibacterial activities against the rest



	1000	100	10
Crude extract	100	0	-10
$CHCl_3$ fraction	100	23.33	6.66
EtOAc fraction	100	16.66	-26.66
n-BuOH fraction	100	23.33	3.33
Aqueous fraction	-3.33	-30	-13.33

Fig. 1: Phytotoxic activities of crude methanolic extract and fractions of *C. murale*

of the pathogens. The Table 2 also indicates that $CHCl_3$ fraction showed good inhibition (60.00%) against *E. coli* and moderate inhibition (46.51%) against *S. aureus,* while it was devoid of any antibacterial inhibition against the rest of tested bacteria. The EtOAc fraction also displayed moderate inhibitory activities (46.66%) and (51.51%) against *E. coli* and *S. flexenari* respectively, while it was ineffective against the rest of the bacteria. The *n*-BuOH fraction exhibited weak inhibition (33.33%) against *E. coli* and moderate inhibitions (41.93%) and (48.00%) against *B. subtilis* and *P. aeruginosa,* respectively. However, this fraction did not display any inhibitory activities against the rest of the tested bacteria. The aqueous fraction exhibited good inhibition (63.63%) against *S. flexenari* and weak inhibition (20.90%) against *S. aureus,* while it was devoid of any inhibitory activities against the rest of the tested pathogens.

The Insecticidal activities were checked against *Tribolium castaneum, Rhyzopertha dominica* and *Callosbruchus analis*. Results in the Table 3 shows that $CHCl_3$ fraction displayed mild activity against *Rhyzopertha dominica* (25%) and *Callosbruchus analis* (20%). The EtOAc fraction showed moderate activity against *Callosbruchus analis* (50%). The *n*-BuOH fraction also exhibited mild activity against *Tribolium castaneum* (30%) and *Callosbruchus analis* (26%). While in rest of the cases no activities were observed.

Results of the phytotoxic activities of the crude extract and its fractions from *C. murale* were interpreted by analyzing the growth regulation in (%) calculated with reference to the negative control. Paraquate was used as standard inhibitor (0.902 μg ml^{-1}). The negative values

Table 1: Antifungal activities of crude methanolic extract and fractions of *C. murale*

Name of fungi	-ve control linear growth (mm)	Crude methanolic extract		$CHCl_3$ fraction		EtOAc fraction	
		Linear growth (mm)	Inhibition %	Linear growth (mm)	Inhibition %	Linear growth (mm)	Inhibition %
Trichophyton longifusus	100	30	70	25	75	20	80
Candida albicans	100	100	0	100	0	50	50
Aspergilus flavus	100	100	0	100	0	100	0
Microsporum canis	100	70	30	35	65	60	40
Fusarium solani	100	100	0	100	0	60	40
Candida glaberata	100	100	0	43	57	50	50

Table 1: Continue

Name of fungi	-ve control linear growth (mm)	*n*-BuOH fraction		Aqueous fraction		Standard drugs (mm)	
		Linear growth (mm)	Inhibition %	Linear growth (mm)	Inhibition %	Name	MIC(μg ml^{-1})
Trichophyton longifusus	100	10	90	35	65	Miconazole	70
Candida albicans	100	45	55	100	0	Miconazole	110.8
Aspergilus flavus	100	100	0	100	0	Amphotericin-B	20.0
Microsporum canis	100	55	45	100	0	Miconazole	98.4
Fusarium solani	100	90	10	100	0	Miconazole	73.2
Candida glaberata	100	100	0	100	0	Miconazole	110.8

Table 2: Antibacterial activities of crude methanolic extract and fractions of *C. murale*

Name of bacteria	Zone of inhibition of standard (imipinem)	Crude methanolic extract		$CHCl_3$ Fraction		EtOAc Fraction		*n*-BuOH Fraction		Aqueous Fraction	
		Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)	Zone of inhibition (mm)	Inhibition (%)
Escherichia coli	30	15.00	50.00	18.00	60.00	14.00	46.66	10.00	33.33	_	0.00
Bacillus subtilis	31	18.00	58.06	_	0.00	_	0.00	13.00	41.93	_	0.00
Shigella flexenari	33	_	0.00	_	0.00	17.00	51.51	_	0.00	21.00	63.63
Staphylococcus aureus	43	_	0.00	20.00	46.51	_	0.00	_	0.00	9.00	20.9
Pseudomonas aeruginosa	25	16.00	64.00	_	0.00	_	0.00	12.00	48.00	_	0.00
Salmonella typhi	41	_	0.00	_	0.00	_	0.00	_	0.00	_	0.00

Table 3: Insecticidal activities of crude methanolic extract and fractions of *C. murale*

Name of insect	Mortality (%) standard drug [permethrin (coopex)] (235.71 μg cm^{-2})	Crude extract (1571.33 μg cm^{-2})		$CHCl_3$ fraction (1571.33 μg cm^{-2})		EtOAc fraction (1571.33 μg cm^{-2})		*n*-BuOH fraction (1571.33 μg cm^{-2})		Aqueous fraction (1571.33 μg cm^{-2})	
		-ve control	Sample	-ve control	Sample	-ve control	Sample	-ve control	Sample	-ve control	Sample
Tribolium castaneum	100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.00	0.00	0.00
Rhyzopertha dominica	100	0.00	0.00	0.00	25.00	0.00	0.00	0.00	0.00	0.00	0.00
Callosbruchus analis	100	0.00	0.00	0.00	20.00	0.00	50.00	0.00	26.00	0.00	0.00

show the growth promotion of the plant. The results (Fig. 1) showed that the crude extract has remarkable phytotoxic activity against *Lemna acquinoctialis* welv. at highest concentration (1000 μg ml^{-1}) and caused complete (100%) inhibition of the plant growth. However, it was completely inactive (0.00%) at (100 μg ml^{-1}) concentration while at further lower concentration (10 μg ml^{-1}) it rather promoted the growth of *Lemna acquinoctialis* by 10%. The $CHCl_3$ extract also showed excellent inhibition (100%) of *Lemna acquinoctialis* at highest concentration (1000 μg ml^{-1}) however, it displayed a weak phytotoxic activity at lower concentrations (23.33% at 100 μg ml^{-1}) and (6.66% at 10 μg ml^{-1}). The EtOAc fraction of the crude extract of *C. murale* also displayed maximum inhibition (100%) of *L. acquinoctialis* at highest concentration (1000 μg ml^{-1}). The lower concentration (100 μg ml^{-1}) did not show remarkable phytotoxic activity and inhibited the growth of the *L. acquinoctialis* by only 16.66%. While at the lowest concentration tested (10 μg ml^{-1}) it promoted the growth of *Lemna acquinoctialis* by 26.66%. Following the same pattern of activity the *n*-BuOH fraction also showed maximum inhibition (100%) to *Lemna acquinoctialis*. The same fraction at lower concentration (100 μg ml^{-1} and 10 μg ml^{-1}) did not show a significant phytotoxic activity and caused inhibition by only 23.33 and 3.33%, respectively. The aqueous fraction was unique in a sense that it promoted the growth of *Lemna acquinoctialis* at all tested concentrations. It promoted the growth of the plant by 3.33, 30.00 and 13.33%, respectively at concentrations of 1000, 100 and 10 μg ml^{-1}.

The crude extract and its fractions from the *C. murale* were also screened for Brine Shrimp cytotoxicity studies but did not display any significant activity in this bioassays.

It is concluded from this study that all the tested samples exhibited well to excellent (65–90%) antifungal activity against the *Trichophyton longifusus*, while none of them displayed any activity against *Aspergilus flavus*. In rest of the cases all samples showed good activity against different pathogens in antifungal bioassay.

The crude methanolic extract and its various fractions of *C. murale* also exhibited mild to moderate inhibitory activities against different bacteria, while they were devoid of any antibacterial activities against other pathogens in the same study.

During this study it was observed that no significant insecticidal and brine shrimp cytotoxic activities were displayed by the tested samples.

It was also observed that the crude extract and its various fractions (except aqueous) showed remarkable phytotoxic activity against *Lemna acquinoctialis* welv. at highest concentration (1000 μg ml^{-1}). However, they displayed weak phytotoxic activity at lower concentrations (100 μg ml^{-1}), while at lowest concentration (10 μg ml^{-1}) few of them rather promoted the growth of Lemna *acquinoctialis* welv. The aqueous fraction promoted the growth of *Lemna acquinoctialis* welv. at all tested concentrations.

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