Journal of Medicinal Plants Research Vol. 6(1), pp. 19-23, 9 January, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.435
ISSN 1996-0875 ©2012Academic Journals

Full Length Research Paper

Antifungal and antispasmodic activities of the extracts of *Euphorbia granulata*

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Accepted 9 May, 2011

The dichloromethane and methanolic extracts of the plant *Euphorbia granulata* were investigated for their antifungal, antibacterial, phytotoxic, brine-shrimp cytotoxic, antioxidant, spasmolytic (antispasmodic) and acetylcholinestrase inhibitory activities. The dichloromethane extract showed strong inhibition against *Microsporum canis* (90%) and against *Aspergillus flavus* (50%). Both the extracts inhibited the spontaneous contractions in rabbit jejunum preparations with EC_{50} value of 0.17 and 1.3 mg/mL, respectively and also relaxed the K^+-induced contractions with EC_{50} 0.2 and 2.8 mg/mL, respectively, suggesting a calcium channel blocking activity. However, the extracts did not show antibacterial, phytotoxic, brine-shrimp cytotoxic, antioxidant and acetylcholinestrase inhibitory activities.

Key words: *Euphorbia granulata*, antispasmodic, antifungal.

INTRODUCTION

The genus *Euphorbia* is one of the sixth largest genera of more than 2000 species flowering plants. The plants produce large number of diverse secondary metabolites such as terpenoids (Khan and Malik, 1990; Macro and Sanz, 1997; Appendino et al., 2000), tannins, polyphenols and flavonoids (Yoshida et al., 1994; Amakura et al., 1997). Various species of the genus *Euphorbia* are used for the treatment of cancer, diarrhea and bronchial asthma (Galvez et al., 1993). *Euphorbia tirucalli* is known to possess cytotoxic and molluscicidal activities (Jurberg and Cabral, 1985). Milliamines isolated from the latex of *Euphorbia milli* showed molluscicidal activity (Zani et al., 1993). *Euphorbia royleana* showed anti-inflammatory activity (Bani et al., 2000), whereas *Euphorbia antisyphilitica* exhibited antihepatotoxic activity (Saraf and Dixit, 1996). The flavonoid glycosides and stepposides from aerial parts of *Euphorbia palustris* and *Euphorbia stepposa* have been reported to possess spasmolytic, choleretic and diuretic effects (Bondarenko et al., 1971). *Euphorbia granulata* showed inhibitory effects against Human immunodeficiency virus (HIV-1) protease (Hussein et al., 1999). It is used in folk medicine as anthelmintic, diuretic, purgative and as a blood purifier (Baquar, 1989). In order to explore further medicinal potential, it was subjected to various biological studies and was found to possess antifungal and spasmolytic activities.

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MATERIALS AND METHODS

Plant material

The whole plant of *E. granulata* (5 kg) was collected from Peruwal (District Khanewal), Pakistan. It was identified by Prof. Dr. Altaf Ahmad Dasti, Plant Taxonomist, Institute of Pure and Applied Biology, B. Z. University Multan, where a voucher specimen is deposited (EG-05-98).

Table 1. Antifungal activity of dichloromethane extract of *E. granulate.* Data is mean of three independent experiments.

Name of fungi	Linear growth (mm)		Inhibition (%)	Standard drugs
	Sample	Control		
Candida albicans	100	100	0	Miconazole
Aspergillus flavus	50	100	50	Amphotericin B
Microsporum canis	10	100	90	Miconazole
Fasarium solani	100	100	0	Miconazole
Candida glabrata	100	100	0	Miconazole

Extraction

The shade dried ground plant material was extracted with dichloromethane and methanol at room temperature, concentrated under reduced pressure by rotavapor.

Antifungal assay

The *in vitro* antifungal bioassay of the crude dichloromethane and methanolic extracts was performed by agar tube dilution method (Atta-ur-Rahman et al., 2001). The crude extracts were evaluated against clinical specimens of *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani and Candida glabrata.* A control experiment with test substance (medium supplemented with appropriate amount of dimethyl sulfoxide DMSO) was carried out for verification of the fungal growth. The extracts (24 mg) dissolved in sterile DMSO (1.0 mL) served as stock solution. Sabouraud dextrose agar (SDA) was dispensed (4 mL) into screw cap tubes which were autoclaved at 121°C for 15 min and 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 day 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. Secondly the antifungal test against *Cladosporium cucumerinum* was carried out on thin layer chromatography (TLC) plate. After developing with suitable solvent system, the TLC plates were well dried with an air dryer and sprayed with a conidial suspension of *C. cucumerinum* in nutrition medium and incubated in moist atmosphere for 2 to 3 days. Inhibition of the fungal growth was observed as clear zones on the chromatogram, indicates the presence of antifungal agents (Chaudhary et al., 2001).

Antispasmodic activity

Animals

Rabbits (1.2 to 1.5 kg) of either sex or local breed were used, housed at the Animal House of the Aga Khan University, maintained at 23 to 25°C and given a standard diet and tap water. Rabbits had free access to water, but food was withdrawn 24 h prior to experiment and killed by a blow on the back of the head. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council (1996) and were approved by the Ethical Committee of the Aga Khan University.

Isolated tissue experiments

The spasmolytic activity of the extracts was studied on isolated rabbit jejunum as described previously (Gilani et al., 2007). Respective segments of 2 cm length were suspended in a 10 mL of Tyrode's solution and bubbled with carbogen gas at 37°C. The composition of the Tyrode's solution in mM was KCl 2.68, NaCl 136.9, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, NaH_2PO_4 0.42, $CaCl_2$ 1.8, and glucose 5.55. A resting tension of 1 g was applied to each of the tissues and was kept constant throughout the experiment. Intestinal responses were recorded isotonically using a Bioscience Transducer and Oscillograph. Each tissue was allowed to equilibrate for at least 30 min before the addition of any drug and then stabilized with a sub-maximal concentration of acetylcholine (0.3 µM) and the bath fluid was subsequently replaced with normal Tyrode solution before starting the experiment. Under these experimental conditions, rabbit jejunum exhibited spontaneous rhythmic contractions, allowing testing of the relaxant (spasmolytic) activity directly without the use of any agonist. To assess whether the antispasmodic effect of the extracts was mediated through calcium channel blockade (CCB), high K^+ (80 mM) was used to depolarize the preparations as described by Farre et al. (1991). Addition of high K^+ to the tissue bath produced a sustained contraction. Relaxation of intestinal preparations by the extracts, precontracted with K^+, was expressed as percent of the control response mediated by K^+. Both extracts were screened for their antibacterial activity against *Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Salmonella typhi*; brine-shrimp toxicity; phytotoxicity against *Lemna minor* as described by Atta-ur-Rehman et al. (2001). The antioxidant and acetylcholinestrase inhibitory assay were also carried out in accordance to reported procedures (Cuendet et al., 1997; Marston et al., 2002).

RESULTS AND DISCUSSION

Antifungal assay was done through the growth in the medium containing crude extracts by measuring the linear growth (mm) and growth inhibition (%) with reference to the negative control. The results (Table 1) indicated that dichloromethane extract showed potent fungal inhibition against *M. canis* (90%) and significant fungal inhibition against *A. flavus* (50%) whereas it was found to be inactive against *C. albicans*, *C. glabrata* and *F. solani*. *M. canis*, a zoophilic dermatophyte most commonly produces tinea capitis and tinea corporis. Tinea corporis in patients with advanced HIV infection can extend over large areas of the body (Wright et al.,

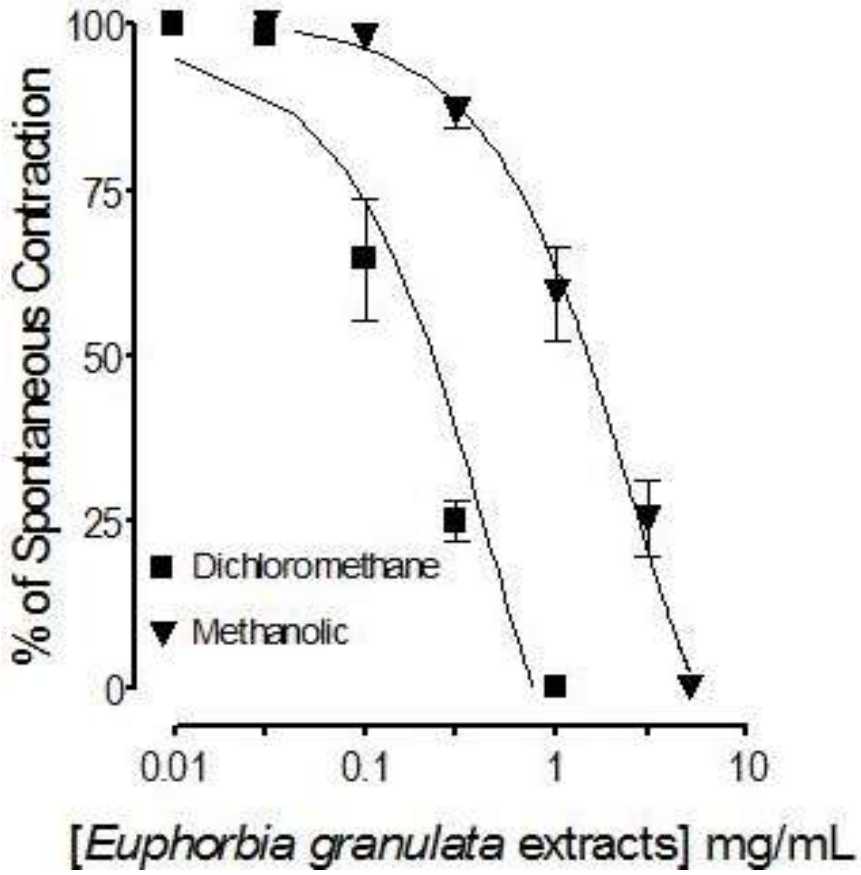

Figure 1. Concentration dependent inhibitory effects of the *E. granulata* dichloromethane and methanolic extracts on spontaneous contractions of isolated rabbit jejunum preparations. Values shown are mean ± SEM, n = 4 for each.

1991). Azole antifungals are generally the most effective agents but are very expensive. *A. flavus* is the common causative organism of all forms of aspergillosis. The major drug of proven value is intravenous amphotericin B. During the past several years, there has been an increasing incidence of fungal infections due to a growth in immunocompromised population such as organ transplant recipients, cancer and HIV/Acquired immune deficiency syndrome (AIDS) patients.

This fact coupled with the resistance to antibiotics and with the toxicity during prolonged treatment with several antifungal drugs (Giordani et al., 2001) has been the reason for an extended search for newer drugs to treat opportunistic fungal infections (Fostel and Lartey, 2000). Plant natural products are of interest as a source of safer and found effective substitutes for synthetically produced antimicrobial agents (Baladrin et al., 1985). The results showed that the crude dichloromethane extract of *E. granulata* has the potential to be an antifungal agent against *M. canis* and *A. flavus*. The discovery of a potent and safe herbal remedy will be a great achievement in fungal infection therapies. Both the extracts inhibited the spontaneous contractions of rabbit jejunum with EC_{50} value of 0.17 and 1.3 mg/mL, respectively (Figure 1). The dichloromethane and methanolic extracts of *E. granulata* relaxed high K^+ (80 mM) -induced contractions with EC_{50} value of 0.2 and 2.8 mg/mL, respectively. At high concentration (> 30 mM), K^+ is known to cause smooth muscle contractions through opening of Voltage-dependent Ca^{++} Channels, (VDCs) allowing influx of extracellular Ca^{++} causing a contractile effect (Bolton, 1979) and a substance causing inhibition of the high K^+-induced contraction is considered an inhibitor of the Ca^{++} influx (Godfraind et al., 1986) (Figure 2). Thus, the spasmolytic effect of the plant extracts, as evident by the relaxation of high K^+ (80 mM) -induced contractions may be due to the calcium channel blockade.

ACKNOWLEDGEMENT

This project was financially supported by B. Z. University, Multan, Pakistan. We are also thankful to HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan for providing technical support.

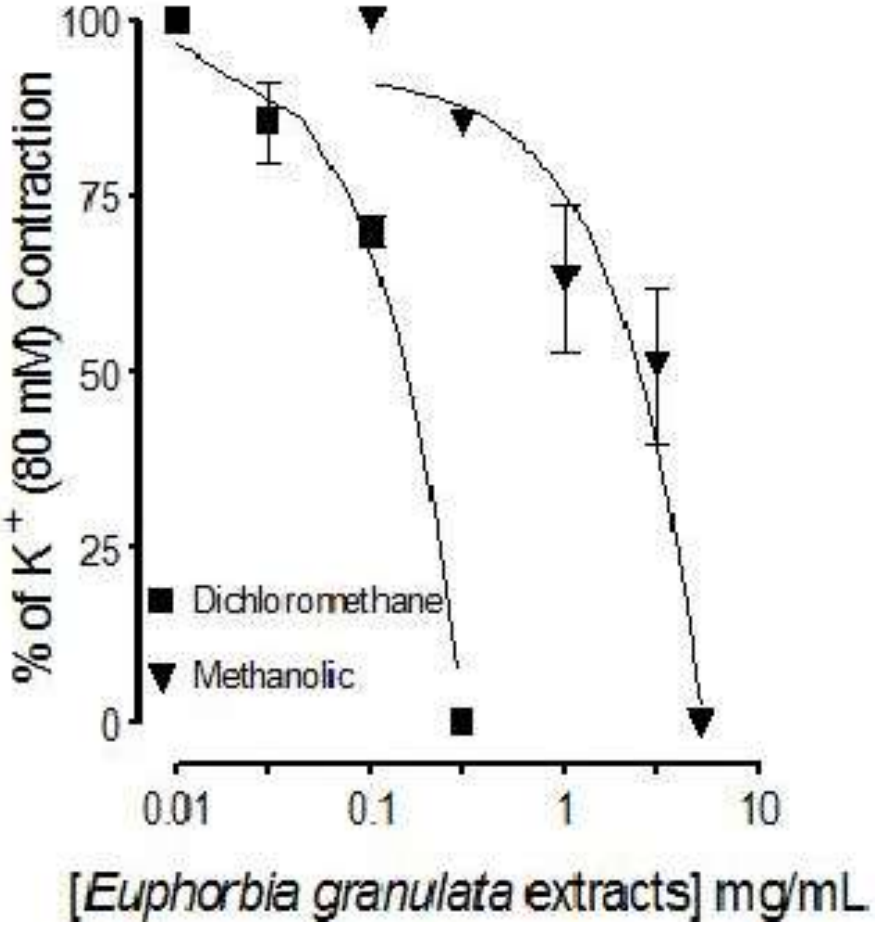

Figure 2. Concentration dependent inhibitory effects of the *E. granulata* dichloromethane and methanolic extracts on high K^+ induced contractions of isolated rabbit jejunum preparations. Values shown are mean ± SEM, n = 3 for each.

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