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Potato Disc Bioassay and Cytotoxic Effect of *Leptadenia pyrotechnica*: Comparative Study of Diverse Extracts

[1]Amal M. Youssef Moustafa, [2]Ahmed I. Khodair and [3]Mahmoud A. Saleh
[1]Department of Chemistry, Faculty of Science, Port-Said University, BP 42523, Port-Said, Egypt
[2]Department of Chemistry, Faculty of Science, Suez Canal University, BP 41522, Ismailia, Egypt
[3]Environmental Chemistry and Toxicology Laboratory, Texas Southern University, Houston, TX, USA

Abstract: Comparative acute toxicity studies of the latex and sequential extracts of *Leptadenia pyrotechnica* (Forsk.) Decne (Asclepiadaceae) were recorded using brine shrimp. The higher toxicities were exhibited in latex; methanol, methanol/dichloromethane (1:1), defatted methanol/dichloromethane (1:1), defatted methanol and dichloromethane extracts. The other extracts; aqueous, alkaloids, ethyl acetate and *n*-butanol exhibited less toxicities compared with the other extracts. The estimated LC_{50} and its 95% confidence limits for these extracts expressed in ppm were: methanol, latex 18.84 (11.22-31.61), methanol/dichloromethane 19.95 (7.76-53.70), defatted methanol/dichloromethane 21.38 (7.24-63.10), defatted methanol 28.19 (16.27-48.81) and dichloromethane 30.90 (11.75-79.43). The anti-tumor activities; potato disc assays of methanol, ethyl acetate and alkaloids extracts showed good activities as anti-tumor agent which represented-49.30,-43.20 an -33.60%, respectively. While latex and aqueous extract represented-30.80 and-28.17%, respectively.

Key words: *Leptadenia pyrotechnica*, Asclepiadaceace, brine shrimp, anti-tumor activity, Latex, ethyl acetate extract, aqueous extract-Potato disk bioassay

INTRODUCTION

Leptadenia pyrotechnica (Forsk.) Decne (Asclepiadaceae) is a plant occurring wild in Sharm El-Sheikh region, Southern Sinai, Egypt. This plant is used in folk medicine to prepare antispasmodic, antiinflammatory, antihistaminic, antibacterial diuretic, expel uroliths, expectorant, gout and rheumatism remedies (Cioffi *et al*., 2006; Panwara and Tarafdarb, 2006; Moustafa *et al*., 2009a). Previous studies led to the isolation of some flavonoids, cardenolides, alkaloids, pregnane glycosides, amino acids, sterols, triterpenoids, fatty acids and fatty alcohols from this plant (Moustafa *et al*., 2007, 2009 b-c, Cioffi *et al*., 2006; Abd El-Ghani and Amer, 2003; El-Hassan *et al*., 2003; Panwara and Tarafdarb, 2006; Noor *et al*., 1993).

A method, utilizing brine shrimp (*Artemia salina* LEACH) (Krishnaraju *et al*., 2006; Poli *et al*., 2006; Ho *et al*., 2005; Pisutthanana *et al*., 2004), is proposed as a simple bioassay for determining LC_{50} values in μg mL^{-1} of extracts. The method is rapid, reliable, inexpensive and convenient as an in-house general bioassay tool (Carballo *et al*., 2002).

Crown gall is a neoplastic disease of plants induced by specific strains of the Gram negative bacterium named *Agrobacterium tumefaciens* (Ohyama *et al*., 1979). The development of a simple anti-tumor pre-screen, using convenient and inexpensive plant tumor systems could, thus, offer numerous advantages as alternatives to extensive animal testing in the search for new anti-cancer drugs (Ferrigni and McLughlin, 1984; Caprioll *et al*., 1992; Schmidt *et al*., 2006; Anderson *et al*., 1992; Mongelli *et al*., 2000).

This paper deals with, a comparison of anti-tumor activity and toxicity using potato disc assay and brine shrimp, respectively, for sequential *Leptadenia pyrotechnica* extracts.

MATERIALS AND METHODS

Plant material: Fresh aerial parts of *L. pyrotechnica* (Asclepiadaceae) were collected in September, during the flowering stage, from Wadi Khashab and Wadi Matzos, Sharm El-Sheikh to El-Tur road, Southern Sinai, Egypt. The identity was established by Dr. Samia Heneidak, department of botany, faculty of science, Suez Canal University. A voucher specimen (Number AMYM-1004) has been deposited in the herbarium of botany department, faculty of science, Suez Canal University, Ismailia, Egypt.

Brine shrimp lethality bioassay: The cytotoxic effect of the extracts total dichloromethane (DCM), total methanol dichloromethane (MeOH/DCM) (1:1), defatted methanol

Corresponding Author: Amal M. Youssef Moustafa, Department of Chemistry, Faculty of Science, Port-Said University, BP 42523, Port-Said, Egypt

dichloromethane (dMeOH/DCM) (1:1), *n*-butanol and aqueous of *L. pyrotechnica* were evaluated by LC_{50} values of the brine shrimp lethality test. While the latex, representing cardiac glycosides and the different extracts; methanol (MeOH), defatted methanol (dMeOH), petroleum ether (pet. ether) representing lipids, total alkaloids and ethyl acetate (EtOAc) representing total flavonoids were tested before(Moustafa *et al*., 2007, 2009b-c). The eggs of brine shrimp were obtained from San Francisco bay Brand, INC., 8239 Enterprice Drive, Newark, U.S.A. The tested samples were dissolved in MeOH and three graded doses, 10, 100 and 1000 μg mL^{-1}, respectively, were used for 5 mL of seawater containing 10 brine shrimp nauplius in each group. The number of survivors was counted in each well after 6 h. Counting of the chronic LC_{50} was begun after 24 h from starting of the test. LC_{50} was determined by probit analysis and Reed-Muench method described by Anderson *et al*. (1991). The experiment was carried out in five replicate and mean LC_{50} values were measured. Control discs were prepared using only MeOH. The negative control solution was simply the same saline solution used to prepare the stock test sample solution. Potassium dichromate was used as standard toxicant and dissolved in artificial seawater, to obtain concentrations of 1000, 100 and 10 ppm.

Anti-tumor Screening of *Leptadenia pyrotechnica* potato disc assay: The potato disc bioassay for the previous plant extracts were carried out. While latex and the different extracts; MeOH, dMeOH and EtOAc were tested before (Moustafa *et al*., 2007, 2009b-c). Tumors were initiated on potato discs (usually Pontiac red or red Russet variations). Fresh, disease-free potato tubers were obtained from local markets. *A. tumefaciens* strain B_6 was maintained on solid slants under refrigeration. Subcultures were grown in 0.8% nutrient broth (Difco) supplemented with 0.5% sucrose and 0.1% yeast extract. Controls were made by the following way: 0.5 mL of DMSO was filtered through Millipores (0.22 μm) into 1.5 mL of sterile distilled water and added to tubes containing 2 mL of the same *A. tumefaciens* strain B6. A Standard solution of Camptothecin was made as follow: 8 mg of Camptothecin was dissolved in 2 mL of DMSO. This solution was filtered through 0.22 μm Millipore filters into a sterile tube. 0.5 mL from this solution was added to 1.5 mL of sterile water and 2 mL of the same broth culture of *A. tumefaciens* strain B_6. A blank solution was made by the following way: 0.5 mL of DMSO was added to 1.5 mL of sterile water. Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. The medium was solidified as required with 1.5% agar (Difco). The results were expressed as + r-percentages versus the number of tumors on the control discs; inhibition was expressed as a negative percentage and stimulation was expressed as a positive percentage. Significant activity was indicated when two or more independent assays gave consistent negative values of ca. 20% or greater inhibition.

RESULTS AND DISCUSSION

LC_{50} determinations: Referring to the data in Table 1 and Fig. 1, 2, the data of mortality rates, point out that, with concentration1 000 ppm, the extracts; DCM, MeOH/DCM and MeOH, beside latex exhibited high mortality; 100%. While the other extracts; dMeOH / DCM, dMeOH, pet. ether, aqueous, alkaloids, EtOAc and *n*-butanol represented 99.1, 98.10, 97.00, 93.55, 93.26, 90.59 and 87.34%, respectively. Moreover, with concentration 100 ppm, latex and the extracts; MeOH, MeOH/DCM, dMeOH/DCM, DCM and dMeOH, pet. ether exhibited the high mortalities; and represented 94.12, 98.63, 91.18, 87.14, 80.95, 79.41 and 73.53%, respectively. While, the other extracts; aqueous, alkaloids and EtOAc, represented less percentages, 62.32, 58.21 and 52.24%, respectively. With concentration 10 ppm, latex and the extracts; MeOH, MeOH/DCM and dMeOH, exhibited the higher mortalities in comparison with the other detected extracts which represented 33.33, 45.10, 32.14 and 25.00%, respectively. While the others, pet. ether, DCM, aqueous, alkaloids, EtOAc and *n*-butanol represented the lower percentages, 22.10, 20.97, 17.11, 14.10, 10.98 and 4.71%, respectively.

The high toxicity was exhibited in MeOH, latex, MeOH/DCM, dMeOH/DCM, dMeOH, DCM and pet. ether extracts. The other measured extracts, aqueous, alkaloids, EtOAc and *n*-butanol exhibited less toxicity

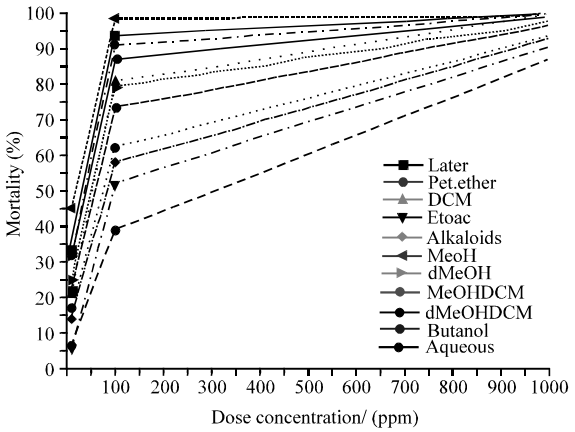

Fig. 1: Relationship between dose concentration of diverse extracts of *L. pyrotechnica* in ppm and the mortality percent

Table 1: Mortality of brine shrimp at various concentration of the different extracts of *L. pyrotechnica*

Plant extract	Dose (ppm)	Dosage (log dose)	Dead	Alive	Accum. dead	Accum. alive	Ratio dead: total	Mortality(%)	LC_{50}ppm
Latex	1000	3	50	04	114	0	114/114	100.00	18.84
	100	2	46	4	64	4	64/68	94.12	
	10	1	18	32	18	36	18/54	33.33	
pet. ether	1000	3	47	3	97	3	97/100	97.00	35.48
Extract	100	2	35	15	50	18	50/68	73.53	
	10	1	15	35	15	51	15/68	22.10	
DCM extract	1000	3	50	0	101	0	101/101	100.00	30.90
	100	2	38	12	51	12	51/63	80.95	
	10	1	13	37	13	49	13/62	20.97	
EtOAc	1000	3	42	8	77	8	77/85	90.59	89.13
Extract	100	2	26	24	35	32	35/67	52.24	
	10	1	9	41	9	73	9/82	10.98	
Alkaloids	1000	3	44	6	83	6	83/89	93.26	63.09
Extract	100	2	28	22	39	28	39/67	58.21	
	10	1	11	39	11	67	11/78	14.10	
MeOH/DCM	1000	3	50	0	112	0	112/112	100.00	19.95
	100	2	44	6	62	6	62/68	91.18	
	10	1	18	32	18	38	18/56	32.14	
dMeOH/DCM	1000	3	49	1	110	1	110/111	99.10	21.38
	100	2	42	8	61	9	61/70	87.14	
	10	1	19	31	19	40	19/59	32.20	
MeOH Extract	1000	3	50	0	122	0	122/122	100.00	11.89
	100	2	49	1	72	1	72/73	98.63	
	10	1	23	27	23	28	23/51	45.10	
dMeOH	1000	3	48	2	102	2	102/104	98.10	28.19
Extract	100	2	38	12	54	14	54/68	79.41	
	10	1	16	34	16	48	16/64	25.00	
n-Butanol	1000	3	40	10	69	10	69/79	87.34	169.82
Extract	100	2	25	25	29	35	29/74	39.19	
	10	1	4	46	4	81	4/85	4.71	
Aqueous	1000	3	44	6	87	6	87/93	93.55	53.09
Extract	100	2	30	20	43	26	43/69	62.32	
	10	1	13	37	13	63	13/76	17.11	

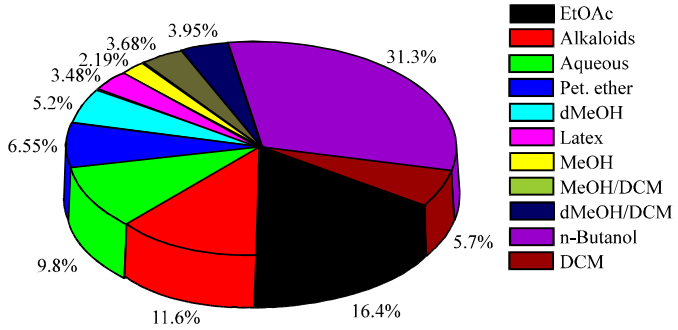

Fig. 2: Relative percentages of LC_{50} of diverse extracts of *L. pyrotechnica*

compared with the other extracts. Moreover, the estimated LC_{50} and its 95% confidence limits for these extracts expressed in ppm were: MeOH 11.89 (11.23-21.05), latex 18.84 (11.22-31.61), MeOH/DCM 19.95 (7.76-53.70), dMeOH/DCM 21.38 (7.24-63.10), dMeOH 28.19 (16.27-48.81), DCM 30.90 (11.75-79.43), pet. ether 35.48 (19.89-63.29), aqueous extract 53.09 (28.73-98.09), alkaloids 63.09 (34.38-115.79), EtOAc 89.13 (22.39-389.05) and n-butanol 169.82 (38.90-562.34).

We find that the EtOAc, alkaloids, aqueous and *n*-butanol extracts are less toxic than the others because the nature of components present in that extracts, almost are flavonoids, alkaloids, carbohydrates, glycosides and saponins, respectively. These types of compounds are less toxic than rest of the compounds detected in this plant (Moustafa *et al*., 2007, 2009b-c). On the other hand, MeOH/DCM, dMeOH/DCM, MeOH, dMeOH and latex contain almost the previous compounds in addition to lipids and cardenolides. Therefore, the toxic effect of that extracts is high.

Potato disc assay: The results obtained from the potato disc assay, Table 2, showed that the MeOH, EtOAc and alkaloids extracts recorded high activities as anti-tumor

Table 2: Inhibition of Tumor Development by *L. pyrotechnica* Fractions on Potato

Fraction	Inhibition %
MeOH extract	-49.30
EtOAc extract	-43.20
Alkaloids extract	-33.60
Latex	-30.80
Aqueous extract	-28.17

Each tested on 5 plates with 5 discs/plate

agent which represented -49.30, -43.20 and -33.60%, respectively. While latex and aqueous extract represented -30.80 and -28.17%, respectively.

The relatively anti-tumor activity of *L. pyrotechnica* could be attributed mainly to its flavonoid constituents and/or alkaloids and to a lesser extent for its cardenolides.

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