Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2011, 1 (1): 51-56

Phytochemical investigation and antitumour activity of *Euphorbia hirta* Linn

Sandeep B. Patil[1]* and Chandrakant S. Magdum[2]

[1]*Appasaheb Birnale College of Pharmacy, South Shivaji Nagar, Sangli, Maharashtra, India*
[2]*Rajarambapu college of Pharmacy, Kasegaon, Sangli, Maharashtra, India*

ABSTRACT

Euphorbia hirta L. (Family: Euphorbiaceae) is a small herb which grows throughout the hotter part of India. The ethanol, chloroform and pet ether extract of Euphorbia hirta L. showed the positive test for tannin, saponin, alkaloids, flavonoids. Antitumour activity of the aerial part of Euphorbia hirta L. has been evaluated against EL-4 cell line (S.C.) in Swiss albino mice. A significant enhancement of mean survival time and reduction of solid tumor mass of Euphorbia hirta L. treated tumour bearing mice was found with respect to control group due to the presence of flavonoids.

Key words: Antitumour activity, EL-4 cell line, *Euphorbia hirta* L.

INTRODUCTION

During the past decades, a exponential increase in exports of medicinal herbs attests worldwide interest in herbal products as well as in traditional health care system. All over the world in last decade, the large number of pharmaceutical industries have been massively invested on pharmacological, clinical and chemical arena with the aim to discover more potent and beneficial natural drugs. The outcome of these efforts were laid down the farmers to initiate commercial cultivation and production of medicinal herbs. Specially in India, geographical and environmental conditions widens the scope of pharmaceutical and phytochemical industries [1].

In view of the farmers most neglected, pan-tropical weed *Euphorbia hirta* L, traditionally used to treat worm infestations, communicable diseases like gonorrhea, jaundice and various skin infections like pimples [2]. The fresh milky latex is applied to wounds, warts and in sprains and inflammation, miscarriage, epilepsy, maggots in wounds and irregular growth of teeth [3]. The aerial parts of plant are qualitatively well investigated for presence of flavonoids (euphorbianin, leucocyanidol, camphol, quercitrin and quercitol) [4,5,6] polyphenols (gallic acid, myricitrin, 3,4-di-O-galloylquinic acid, 2,4,6-tri-O-galloyl-D-glucose, 1,2,3 ,4,6-penta-O-galloyl-β- D-glucose [7,8]. Tannins (Euphorbins A, B, C, 0, E) [9]. Triterpenes and phytosterols (β-Amyrin, 24-methylenecycloartenol, and β-Sitosterol) [10]. Alkanes (Heptacosane, n-nonacosane) [11]. The plant has been reported for antiamoebic and antispasmodic[12], antidiarrhoeal[13], anti-inflammatory [14] and antibacterial activities [15].

These phytoconstituents are found to be reported as a potent tissue growth inhibitors. This fact compels us to screen the aerial parts for its potency against various cell lines in vivo.

MATERIALS AND METHODS

Plant material

The plant of *Euphorbia hirta* L. was collected from Sangli region. The plant was authenticated by Dr. U. S. Yadav, Dept. of Botany Willingdon College Sangli. Specimen vouchers were also kept with number W.E.T.001 for future reference. The whole plant was dried in shade. The powder was then packed individually into Soxhlet apparatus and subjected to hot continuous percolation using pet. ether, chloroform, ethanol (95% v/v) as solvent successively. The extract was concentrated under vacuum.

Animals used

Swiss albino mice (20 to 25 g) were used throughout the study. They were housed in average microlon boxes and were given standard laboratory diet and water *ad libitum.* The experements were performed in accordance with the guidelines established by the European community for the care and use of laboratory animals and approved by the institutional animal ethics committee (IAEC) and conducted according to guidelines of committee for the purpose of the control and supervision on Experiments on animals (CPCSEA), India.

Tumour cell line

The cell line under investigation was EL4 (mouse; T lymphocyte; lymphoma). It was purchased from the National cell culture sciences, Pune. The cells were cultured in DMEM with 4 mM l-glutamine adjusted to contain 1.5g/L Na bicarbonate and 4.5 g/L glucose, 90%; horse serum, 10%.

Drugs and chemicals

5- Fluorouracil (5-FU) from sigma chemical Co., St. Louis, Mo, USA. The other entire reagents used were of analytical reagent grade.

Preliminary phytochemical investigation

Pet. ether, chloroform, ethanol extract of *Euphorbia hirta* L was investigated for phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins,

proteins, triterpenoids. Phytochemical screening of the extracts was performed using the standard procedures [16].

Acute toxicity studies (LD_{50}): Acute toxicity of the extract Euphorbia hirta Linn was evaluated in mice using the up and down procedure (OECD, 2001)

Antitumour activity: Before starting experiment the total cell count of the cancer cell suspension was measured. The solid tumors were induced in Swiss albino mice (five per group) by injecting EL4 cells (1×10^6 cells per animal) subcutaneously. After the tumor inoculation the dose of 200mg/kg of pet ether, chloroform and ethanolic extract of aerial part of *Euphorbia hirta* L. were administered orally 24 hr. every day for 10 day [17]. Similarly standard 5-fluorouracil (20mg/kg) was administered orally 24 h after the tumor inoculation everyday for 10 days. The volume of tumor was measured every third day for 1 month. The solid tumor development was measured with the Vernier Caliper and calculated using the formula, $V = 4/3 \times \pi \times r_1 \times r_2 \times r_3$, Where r_1, r_2 and r_3 are radii along two directions and V is volume in mm^3.

Statistical analysis
The parameters data were evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's t test.

RESULTS AND DISCUSSION

New scientific strategies for the evaluation of natural products with biological activity require the implementation of large-scale screening programs. Preliminary phytochemical screening showed the presence of alkaloids, steroids, flavonoids in all extract of *Euphorbia hirta* L.(Table No.1), The in-vivo anti tumour activity of pet ether, chloroform and ethanol extract from *Euphorbia hirta* L. (dose of 200 mg/kg/day, oral.) was evaluated. Anti-tumour activity of *Euphorbia hirta* revealed that mice in control group (tumour cells + N.S.) had larger size of tumour as compared to mice in standard group (tumour cells + 5-FU). The tumour size of control group increased from 9 th to 30th day (0.3340 mm^3 to 4.8100 mm^3) as compared to Standard group at the end of 18th day. But after 20th day the small size of tumour was noted (0.4172 cm^3) in standard group. Ethanol and chloroform extract of *Euphorbia hirta* L. showed very small size of tumour (0.5220 to 2.1382 mm^3) (0.5168 to 2.6865 mm^3) in this investigation period and thus showed moderate antitumour activity as compared to standard used respectively. Pet ether extract of *Euphorbia hirta* L. failed to reduce the size of tumour. The reliable criterion for judging the value of any anticancer drug is the prolongation of lifespan of the animal [18]. The effect of pet ether, chloroform and ethanol extract on the survival of tumour bearing mice is expressed as mean survival time (MST). It was found to be 21days, 29 days (138.09 %), 32 days (152.38 %) and 34 days (161.90 %) , 40 days (190.47%) for the control group, pet ether extract, chloroform extract and ethanolic extract , 5-Flurouracil (20 mg/kg/day i.p.) respectively. (Table No. 2 , Table No.3). In present study showed antitumor activity of chloroform and ethanolic extracts of *Euphorbia hirta* L. against EL4 cell line in Swiss albino mice significantly. Results of in vivo activity suggested that the isolated flavonoids may have a chemo preventative role in cancer through their effects on signal transduction in cell proliferation and angiogenesis.

Table 1: Preliminary phytochemical screening of *Euphorbia hirta* L. (+ Positive test, - negative test)

Sr. No.	Chemical Test	Inference Chloroform extract	Pet ether extract	Ethanolic extract
1	Test for Steroids			
	1) Salkowaski test:	+	+	+
	2)Liebermann Burchard test:	+	+	+
	3)Liebermann-Burchard reaction:	+	+	+
2	Test for Cardiac Glycosides			
	a) Balget's test:	-	-	-
	b) Keller killiani test	-	-	-
	c) Legal's test	-	-	-
3	Tests for saponin glycosides:			
	Foam test	+	+	+
4	Test for Carbohydrates			
	a) Molisch's test	-	-	-
	b) Barfoed's test	-	-	-
	c) Benedict's test	-	-	-
5	Test for Alkaloids			
	a) Mayer's test	+	+	+
	b) Wagner's test	-	-	-
	c) Hager's test	+	+	+
	d) Dragendorff's test:	-	-	-
6	Test for Flavonoids			
	a) Shinoda's test:	+	+	+
	b) To small quantity of residue, added lead acetate solution.	+	+	+
7	Test for Tannins			
	a) 5% Ferric chloride test:	-	-	+
	a) Gelatin test:	+	-	+

Table 2: Effect of E. hirta extract on the survival of tumour bearing mice (n=10 animals in each group, P< 0.01 Vs control. Days of treatment =9. Values are expressed as mean ± SEM).

Treatment	MST(d)	Increase in life span (%)
Tumour control	21 ± 1.20	100
5- FU (20 mg/kg i.p.)	40 ± 2.10	190.47
Pet ether extract (200mg/kg)	29 ± 1.20	138.09
Chloroform extract (200mg/kg)	32 ±1.20	152.38
Ethanolic extract (200mg/kg)	34 ± 1.19	161.90

Table 3: *In vivo* Anti-tumour Activity of *Euphorbia hirta linn*

No. of days	Volume of solid tumour in mm^3				
	Control (0.5 ml N.S.)	Standard (5 FU of conc. 20 mg/kg)	Ethanolic extract of Euphorbia hirta L (dose = 200 mg/kg)	Pet.ether extract of *Euphorbia hirta* L (dose = 200 mg/kg)	Chloroform extract of Euphorbia hirta L (dose = 200 mg/kg)
3	_	_	_	_	-
6	_	_	_	_	-
9	0.334	_	_	0.2672	-
12	0.5011	_	_	0.4172	-
15	0.877	_	0.522	0.5011	0.5168
18	1.1544	_	0.6264	0.877	0.6845
21	1.8014	0.4172	0.902	1.22	0.8736
24	2.0296	0.4172	1.22	2.1048	1.4084
27	3.5	0.6264	1.6036	2.4054	1.8024
30	4.81	0.902	2.1382	3.0068	2.6865

Acknowledgements

The authors wish to express thanks to Director, NCCS. Pune, for providing cell lines. The authors wish to express their thanks to Principal, Appasaheb Birnale College of Pharmacy, Sangli (India) for encouragement and for providing the necessary facilities and excellent support.

REFERENCES

[1] Panda, H,; Handbook on Medicinal Herbs with Uses. Asia Pacific Business Press, Delhi, **2004**.
[2] Laloo R.C., Kharlukhi L., Jeeva S., and Mishra B.P., *Current Science*, **2006**, 90 ,225-235.
[3] Kirtikar K.R. and Basu B.D.; Indian Medicinal Plants.,I-III Periodical Expert Book Agency, Delhi **1991**.
[4] Gupta D.R., *Bull. Chem. Soc.*, **1966**, 39(11), 2532-2534.
[5] Blanc P. and Bertrand, P. De saqui-Sannes, *Plantes Med. Phytother*; **1972**, 6, 106-109.
[6] Aqil M.,. *Global J. Pure Appl. Sci.* **1999**, 5(3), 371-375.
[7] Chen L., *Zhongguo Zhong Yao Za Zhi*. **1991**, 16(1), 38-39 ().
[8] Yoshida T. and Chen L., *Chem. Pharmaceut. Bull* **1988**, 36(8), 2940 -2949.
[9] Yoshida T., Namba O. and Okuda T., *Tennen Yuki Kagobutsu Toronkai Koen Yoshishu.* **1989**, 31: 601-608.
[10] Martinz V., *Rev. Sociedad Quimica De Mexico.* **1999**, 43(3-4): 103 -105.
[11] Gnecco S., *Boletín de la Sociedad Chilena de Química.* **1996**, 41(3): 229-233.
[12] Tona L., Kambu K., Ngimbi N., Mesia K., Penge O., Lusakibanza M., Cimanga K. De Bruyne, Apers T., Totte S., Pieters J., L. and Vlietinck A..J., *Phytomedicine.* **2000**, 7(1): 31-38.
[13] Tona L. and Kambu K., *Phytomedicine* . **1999**, 6(1): 59-66.
[14] Lanhers M.C., Fleurentin J., Dorfman P., Mortier F. and Pelt J. M., *Planta Med.* **1991**, 57(3): 225-231.
[15] Vijaya K., Ananthan S., and Nalini R., *J. Ethnopharmacol* **1995**, 49(2): 115-118.
[16] Khandelwal KR., Practical Pharmacognosy, 12th ed., Nirali Prakashan, Pune, **2004**.

[17] B. Rajkapoor. *Indian J. Pharmacol* **2004**, 36 (1): 38-40.
[18] Clarkson B.D., Burchenal J. H., *Progress in Clinical Cancer*. **1965,** 1: 625–629.