Invitro anti-tumour studies on *Cnicus wallichi* DC

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Abstract: *Cnicus wallichi DC belonging to the family Asteraceae (Compositae) commonly known as Indian thistle and cirsium wallichi. It is an important medicinal plant indigenous to Nilgiris, Tamilnadu, South India. Since the related species Cnicus benedictus was reported for its anti cancer activities, Invitro screening studies for antitumour activities were carried out for different extracts of Cnicus wallichi DC. Seven different extracts were obtained from the aerial parts of the whole plant by successive solvent extraction and maceration process and subjected for Invitro screening studies. Antitumour study was carried out by short term toxicity studies using Dalton's Lymphoma Ascites (DLA) cells. The ethyl acetate extract of Cnicus wallichi DC showed significant antioxidant activity in all the methods.*

Keywords: *Cnicus wallichi DC, Invitro studies, anti tumour activity.*

Introduction

Cnicus wallichi DC belonging to the family Asteraceae (Compositae) is a sub shrub and erect that occurs usually in Pulney hills, Himalayas, Palani and Nilgiri hills of Tamilnadu, South India. According to the ethno botanical information, the aerial parts of the plant extracts are used externally for cuts, wounds, ulcers ,abscesses and also used as a galactogogue. The roots have cooling effect and are used as a tonic and expectorant. In Nepal, a teaspoonful of pounded roots of the herb is given twice a day to control stomach inflammation[1,2]. The whole plant has a recognized medicinal value. Since the related species *Cnicus benedictus* was reported for its anticancer activities[3] *Invitro* screening studies for antitumour and antioxidant activities were carried out. Antitumour study was carried out by short term toxicity studies using Dalton's Lymphoma Ascites (DLA) cells. *Cnicus wallichi* DC is commonly known as Indian thistle, *Cirsium wallichi*, thistle, buch bucham and Dharabindhak(Hindi)[4]. Sesquiterpenes, flavonoids and steroids were reported as the main chemical constituents in *Cnicus wallichi* DC[5]

Materials and methods

The aerial parts of the *Cnicus wallichi* DC were collected, identified and authenticated by Dr. S. Rajan, Botanist, Survey of medicinal plants and collection unit, Government Arts College, Ooty, Tamilnadu, Southindia. The specimen of the plant No.2526 was preserved in the laboratory of TIFAC CORE HD, JSS College of Pharmacy, Ooty for further reference. The aerial parts of the plant were separately chopped in to small pieces and dried in shade. Then they were passed through sieve no 20 and used for extraction. The dried aerial parts of the plant material (500g) were extracted in a soxhlet apparatus by successive solvent extraction process. Petroleum ether, chloroform, ethyl acetate and successive methanol extracts were obtained. 50% methanol, methanol and aqueous extracts were obtained by maceration[6]. The plant extracts were subjected to Preliminary phytochemical screening for the detection of various plant constituents present, according to standard procedures[7].The results are tabulated in Table 1.

Pharmacological studies

The different aerial part extracts of *Cnicus wallichi* DC were screened for the *invitro* studies such as antitumour activity studies[8].

Invitro anti tumour studies

This study was carried out by short term toxicity studies using Dalton's Lymphoma Ascites (DLA) cells. This test relies on break down in membrane integrity determined by the uptake of a dye such as tryphan blue, erythrosine and nigrosin to which the cell is normally impermeable. DLA cells were cultured in peritoneal cavity of mice by injecting intraperitonially a suspension of DLA cells ($1.0X10^5$ cells/ml). The DLA cells were then with drawn from the peritoneal cavity of the mice between 15 to 20 days with the help of a sterile syringe. The cells were washed with Hanks Balanced Self Solution (HBSS) and the cell count was adjusted to $2X10^6$ cells/ml.

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Table 1 Qualitative phytochemical analysis of various extracts of *Cnicus wallichi* DC

Sl. No	Phyto constituents	Petroleum ether	Chloroform	Ethyl acetate	Successive methanol	50% methanol	Aqueous	Methanol
1	Alkaloids	-	-	-	-	-	-	-
2	Carbohydrates	-	+	+	+	+	+	+
3	Proteins and amino acids	-	-	-	-	-	-	-
4	Steroids	+	+	+	+	+	+	+
5	Glycosides	-	-	-	-	-	-	-
6	Saponins	-	-	-	-	-	-	-
7	Flavanoids	-	-	+	+	+	+	+
8	Tannins and Phenols	-	-	+	+	+	+	+
9	Triterpenoids	+	+	-	-	-	-	+
10	Fixed oils	-	-	-	-	-	-	-

+ Positive - Negative

Table 2 *Invitro* antitumour* studies on *Cnicus wallichi* DC

Sl. No	Name of the Extracts	CTC_{50} values in gm/ml
1	Petroleum ether	275 ± 3.25
2	Chloroform	280 ± 1.18
3	Ethyl acetate	175 ± 2.15
4	Successive methanol	285 ± 2.30
5	50% methanol	310 ± 3.20
6	Aqueous	325 ± 2.31
7	Methanol	350 ± 2.42

*Average of three determinations

The diluted cell suspension was distributed in to Eppendorf tubes. The cells were exposed to drug dilutions and incubated to 37^{0}C for 3 hours. After 3 hours, the dye exclusion test is performed. Equal quantities of the drug treated cells and tryphan blue (0.4%) were mixed and left for a minute. It was then loaded in a haemocytometer and viable and non viable count was recorded within two minutes. If kept longer, live cells also generate and take up color. Viable cells do not take up color, whereas dead cells take up color. The percentage growth inhibition was calculated by using the following formula [8].Results is tabulated in table 2.

% Growth inhibition = 100- Total cells Dead cells / Total cells X 100

Results and Discussion

The preliminary phytochemical analysis of the aerial part extracts of the plant showed the presence of steroids, triterpenoids, phenolic compounds, Flavanoids, tannins and carbohydrates. The ethyl acetate extract of *Cnicus wallichi* DC showed antitumour activity at the concentration of 175 gm/ml. Preliminary phytochemical studies revealed the presence of flavanoids and their glycosides in ethyl acetate extract. The flavanoids are known for their potent antioxidant and antitumour activities. Hence, this extract can be subjected for the *in vivo* studies of anti oxidant and antitumour activities and also for isolating the bio active principles. All the results obtained are mentioned in Table 1 and 2.

References

1.Krithikar. K. R and Basu B. D, Indian Medicinal Plants, , 2nd Ed, 1 (1935) 293.

2.chopra .R.N,. nayar. S.N and chopra .I.C), Glossary of Indian Medicinal plants, Council for Scientific and Industrial Researach, New Delhi, (1996) 158.

3.Fyson, P.F, The flora of the Nilgiri and Pulney hill tops ,1 (1974) 246-247.

4.Kataria. H. C, Phytochemical investigataion of Medicinal Plants *cnicus wallichi* DC and *Cnicus benedictus*, *Asian journal of Chemistry* ,7 (1995) 227-228.

5.Kokate. C. K), Practical Pharmacognosy, Vallabh Prakashan Publishers, New Delhi, 4th Ed, (1999)123-124.

6.Raaman. N, Phytochemical techniques, New India Publishing agency, New Delhi (2006) 5-15.

7. Skeham. P., et al.,. New calorimetric cytotoxic assay for anti cancer drug screening, *Journal of nat cancer* ,82(32) (1990)1107-1123.