Rashmi Kalia et al /J. Pharm. Sci. & Res. Vol. 8(11), 2016, 1262-1265

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

Antitumor Activity of Aqueous Leaf Extracts of Different Cultivars of *Chrysanthemum morifolium* R. using Potato Disc Tumor Assay.

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Abstract

Plants are considered to be the main source of biologically active compounds. About 50% of prescribed medicines in Europe and USA are estimated to arise directly or indirectly from plants. Various bioactive compounds have been derived from different parts of plants which show antibacterial, antifungal, antioxidative, antitumor, antimutagenic and anticancer properties. Chrysanthemum, a common ornamental and also a medicinal plant, is a good source of retinol, thiamine, niacin, folic acid, pantothenic acid, ascorbic acid, calcium, magnesium, potassium, iron and phosphorus. It is rich in flavonoids like luteolin, apigenin and acacetin, choline. The plant is also said to possess antibacterial, antioxidative, antifungal, antitumor antiaging and insecticidal properties. Keeping this in mind, the present study was carried out to estimate the antitumor potential of aqueous leaf extracts of ten different cultivars of *Chrysanthemum morifolium* using potato disc tumor assay. It was noticed that Yellow Coin cultivar of Chrysanthemum exhibited maximum (69.85%), while Cameo cultivar showed minimum (56.55%) inhibition in the induction of tumors in the potato disc tumor assay. Overall results supported that aqueous leaf extracts of *C. morifolium* are potential source of antitumor agents that can be explored for further drug development for tumor treatment in humans.

Key words: Antitumor, Chrysanthemum, Potato disc tumor assay

INTRODUCTION

Today, despite the worldwide efforts, cancer remains one of the most dreadful life threatening diseases. Moreover, use of chemical based medicines has not yielded the results up to the expectations in spite of high cost involved in the development of these drugs. Therefore, there is a need to develop affordable and effective anticancer drugs [1]. In the last few decades, interest in natural and traditional medicine has increased tremendously [2, 3]. Natural products have received great attention over the last 50 years as potential cancer preventive and therapeutic agents. It has been suggested that aqueous and ethanolic extracts of plants which are used in allopathic and ayurvedic medicines are potential sources of antitumor and antiviral agents [4]. Approximately 60% of the anticancer drugs being used currently are derived either from plants or plant products [5] The National Cancer Institute (NCI) has shortlisted 35000 plants with anticancer activity [6]. The anticancer properties of some of the plants are still being explored.

Chrysanthemum, an important member of the family asteraceae, is known for its beautiful flowers. The flowers are borne in inflorescence and are of different shapes, sizes and colors. Chrysanthemums in addition to their ornamental importance also have culinary, environmental, insecticidal and medicinal uses that are known to man since centuries. Although huge literature is available on bioactivities of flowers of species [7-11], little is known about the activities of the leaves of chrysanthemum.

Different bioassays offer vast number of advantages for screening of medicinal plant extracts for different bioactivities eg. antitumor, antibacterial, antioxidative, antigenotoxic etc. Among various bioassays, inhibition of tumors induced by *Agrobacterium tumefaciens* on potato discs is an assay which is based on antimitotic activity of the plants/plant extracts [12]. Potato disc tumor assay is an efficient assay to screen antitumor activity of the crude extracts of various plants, regardless of the mode of inhibitory action on tumor formation. Many scientists have used this assay to assess the antitumor potential of various plants [13-16]. Validity of this bioassay has been predicted on the basis of the fact that certain tumorigenic mechanisms are similar in animals and plants [17]. According to Kempf et al. [18] pathogenic strategy of *A. tumefaciens* in plants is similar to *Bartonella henselae*, a tumor causing bacterium in humans.

As many bioactivities of chrysanthemum flower have already been studied, the present study was carried out to specifically study the antitumor potential of the aqueous leaf extracts of 10 different cultivars of *Chrysanthemum morifolium.*

MATERIALS AND METHODS

Collection of the sample

Fresh and disease free leaves of 10 cultivars (Basanti, Bravo, Cameo, Flirt, Garden Beauty, Jaya, Sadbhawna, Winter Queen, Yellow Charm and Yellow Coin) of *Chrysanthemum morifolium* were collected from the plants procured from Punjab Agricultural University, Ludhiana, Punjab.

Preparation of the extract

Chrysanthemum leaves of all cultivars were taken separately, washed thoroughly and kept in shade until completely dried. The dried leaves were powdered using a grinder. 20 g leaf powder was suspended in 200 ml of water and kept in incubator shaker at 120 rpm and 50^{0}C for 16 h. The suspension was then filtered and filtrate was considered as 100% Chrysanthemum leaf extract. The filtrates were then stored at -20^{0}C for further use.

Estimation of antitumor properties of the aqueous extracts

Potato Disc Tumor Assay was used to study the antitumor effects of the aqueous leaf extracts using protocol suggested by Coker et al. with certain modifications [19]. *Agrobacterium tumefaciens* strain Microbial Type Culture Collection (MTCC) Number 431 was procured from IMTECH, Chandigarh. The bacterial culture was then grown in LB (Luria Broth) medium and incubated in an incubator shaker at temperature 28 ^{0}C for 16 h. Small fresh red skinned potatoes were procured from the local market and washed under running tap water for 2-3 min. The potatoes were then peeled with a sterile knife and small discs of 0.5 cm thickness and 1.0 cm diameter were cut with the help of a sterile cork borer. The discs were then disinfected using 10% bleach solution. Autoclaved agar solution (1.5%) was poured in the Petri plates and solidified. 5 discs were then gently placed on solidified agar in each plate. 50 µl inoculum was prepared by mixing equal quantities of bacterial culture and chrysanthemum leaf extract and poured over each disc of the plate. The process was repeated for extracts of different cultivars of chrysanthemum. 25 µl of bacterial culture and 25 µl sterile water was used as control. The experiment was carried out in triplicate. The lids of the Petri plates were sealed with the help of parafilm and then the plates were incubated in dark at 28 ^{0}C for 12 days. After incubation, discs were stained with Lugol stain (5% KI + 5% Iodine). The tumors were scored with the help of a stereoscope with magnification of 25X. Tumor inhibition was calculated using following formula

$$\text{Tumor inhibition (\%)} = 100 - \frac{\text{Number of tumors present in the sample}}{\text{Number of tumors in the control}} \times 100$$

Statistical analysis

The data were presented as mean ± SE and analyzed using one way analysis of variance (ANOVA) at $p \leq 0.05$.

RESULTS AND DISCUSSION

Present study was successful in revealing antitumor potential of the aqueous leaf extracts of chrysanthemum. Table 1 and Fig 1 show number of tumors and percent inhibition of potato disc tumor induction by aqueous leaf extracts of 10 different cultivars of *C. morifolium.* Among different cultivars studied, Cameo showed maximum numbers of tumors (11.27±0.78 SE) while minimum numbers of tumors were observed with Yellow coin (7.82±0.52SE). The number of tumors present in the control was 25.94±0.90. These results indicated that the Yellow Coin cultivar of chrysanthemum showed maximum antitumor effect (69.85%) followed by Sadbhawna (68.42%), Bravo (68.31%), Yellow charm (65.61%), Flirt (65.33%), Jaya (62.29%), Garden beauty (61.33%), Winter queen (59.56%) and Basanti (57.74%). The minimum antitumor effect was observed for Cameo cultivar (56.55%).

Table 1. Antitumor potential of aqueous leaf extracts of 10 different cultivars of *Chrysanthemum morifolium* in potato disc tumor assay.

S.No.	Cultivar	No. of tumors (Mean ±S.E)	Percent inhibition
1	Control	25.94±0.90	--
2	Basanti	10.96±0.94[a]	57.74
3	Bravo	8.22±0.40[a]	68.31
4	Cameo	11.27±0.78[a]	56.55
5	Flirt	8.94±0.67[a]	65.53
6	Garden beauty	10.03±0.64[a]	61.33
7	Jaya	9.78±0.44[a]	62.29
8	Sadbhawna	8.19±0.56[a]	68.42
9	Winter queen	10.49±0.36[a]	59.56
10	Yellow Charm	8.92±0.30[a]	65.61
11	Yellow coin	7.82±0.52[b] HSD- 3.184, F- Ratio 65.68[*]	69.85

Data shown are Mean ±SE of three experiments. [*] Significant at $p \leq 0.05$.
Means followed by the same letter are not significantly different using HSD multiple comparison test.

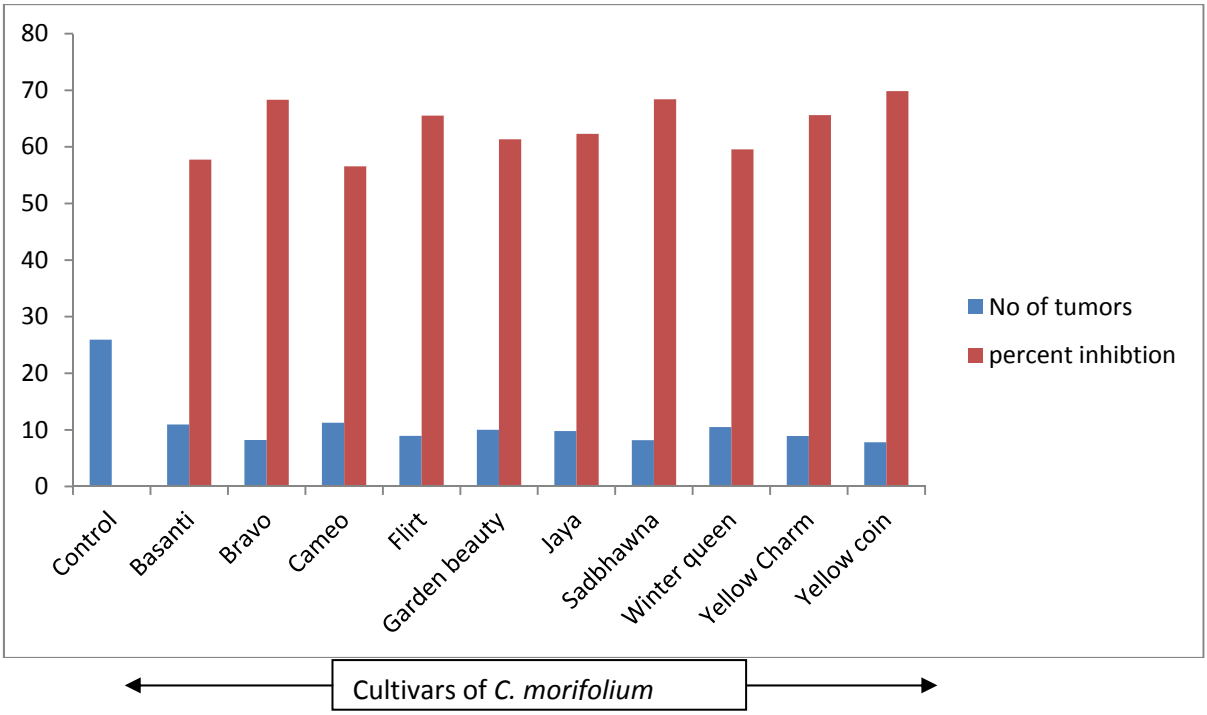

Fig1. No of tumors and percent inhibition of tumor formation in PDTA following treatment with aqueous leaf extracts of different cultivars of *Chrysanthemum morifolium* .

Crown gall is a neoplastic disease of plants which affects dicotyledons and many gymnosperms. Causative organism is specific strain of gram negative bacterium *Agrobacterium tumefaciens* which has large Ti Plasmid (tumor inducing plasmid) that carries genetic information to transform normal plant cells into autonomous tumor cells in a short period of time [20]. In the field of cancer fighting, it is always considered better to prevent cancer than to cure it [21], hence food plants and medicinal plants containing anticancer agents are considered very important in the field of biomedical research [22,23]. For many plants there is no relevant literature available, so the antimutagenic/ antiproliferative/ antitumor activities of such plants need to be studied using appropriate pharmacological methods. The potato disc tumor assay is a fast, simple and inexpensive method to screen the antitumor potential of different products including plant products [24]. Many bioactive compounds have been isolated from chrysanthemum such as pyrethroids, phenolics, sterols, luteolin, quinic acid, and polyacetylenic compounds [25]. Antitumor activity of flavonoids has been well documented. Luteolin, which is the main flavonoid present in chrysanthemum is supposed to be the main reason for the antitumor activity of this plant. It inhibits the activity of topoisomerases which are very important in the synthesis of DNA [26, 27]. Water extract of chrysanthemum also possesses inhibitory effects against several free radicals [28]. More than 50 terpenoids isolated from chrysanthemum have been shown to possess antitumor potential against many cancer cell lines [29, 30]. However, as terpenoids are insoluble in water, so they are unlikely to be the active compounds in the water extract of chrysanthemum. Many flavonoids are also present in chrysanthemum. As flavanoids are soluble in water, they are believed to be present in the water extract of chrysanthemum [31- 33]. The antitumor activity observed in the present study can be attributed to the presence of flavonoids

CONCLUSION

Ever since its establishment, potato disc tumor assay is widely used to evaluate antitumor properties of plants. It is a fast, convenient, inexpensive assay, especially, for assessing antitumor agents in plants. Among innumerable advantages, this assay offers an alternative to more expensive animal testing in preliminary screening for anticancer drugs. Although flowers of chrysanthemum have been used by people for the cure of different ailments including cancer, little is known about the antitumor potential of its leaf extracts. From the results of the present study, it is clearly indicated that aqueous extracts of leaves of *C. morifolium* possess remarkable antitumor potential. As *chrysanthemum morifolium* extracts are already in use for the cure of different ailments, these findings suggest to further explore the leaf extracts of *Chrysanthemum morifolium* for antitumor potential in animal assays to development of novel antitumor drugs from leaves which can be utilized in therapeutics.

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