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Research Article

ANTICANCER ACTIVITY OF *CURCUMA KARNATAKENSIS* – A REAR AND ENDEMIC PLANT USING SULFORHODAMINE-B ASSAY

SUJATHA BS*, TEJAVATHI DH

Department of Botany, Bangalore University, Bengaluru, Karnataka, India. Email: shivasuji05@gmail.com

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ABSTRACT

Objectives: The objectives are to study the anticancer activity of *Curcuma karnatakensis* collected from different localities using sulforhodamine-B (SRB) assay.

Methods: Anticancer activities of two plant samples were detected on human breast cancer cells (MDA-MB-468) by following SRB assay method. We have used two plant samples collected from two different localities, we specifically used 1.5 mg and 3 mg of extract and study was done for 24 h and 48 h duration, optical density was read at 540 nm using microplate reader, and percentage inhibition of cancer cell growth was recorded. The low concertation of the plant sample was found to be very active and effective than the other in less duration of time.

Results: Anticancer activity of both the samples was analyzed for different time durations with different concentrations and it was found that sample B has not shown anticancer activity at both the treatments. The difference in their activity may be due to the difference in growing areas and difference in soil type where it is growing with different mineral compositions.

Conclusion: The present work on this rare species gives basic information regarding their anticancerous behavior in low dosages and this is the stepping stone to study further about use of this plant in the medicinal field.

Keywords: Anti-cancer, MDA-MB-468, Sulforhodamine-B assay.

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INTRODUCTION

Cancer is one of the deadly diseases; it is the third leading cause of death in the world after some infectious diseases and cardiovascular diseases [1]. Prevention is considered a superior approach in dealing with this disease. About 60–70% of drugs are obtained from natural plant products to treat cancer [2]. Hence, consuming the plants having anticancer properties is followed in most of the countries. The plant extracts such as onion, *Tulasi*, and *vinca* roses are used in the treatment of cancer. The important among them are curcumin from turmeric, tea polyphenols from tea, sulforaphane from broccoli, genistein from soybean, diallyl sulfide from garlic, and resveratrol from grapes. Anticancerous property of *Catharanthus roseus* is due to the two important components called vincristine and vinblastine [3]. *Nothapodytes foetida* contains three important compounds which are very useful in treating cancer disease. Compounds are acetylcamptothecin, camptothecin, and scopoletin. These are useful in inhibiting DNA topoisomerase in cancerous cell [4].

There is a constant struggle in globe in curing and preventing cancer. Cancer initiates when there is a genetic change within the normal cell cycle. The cells start to grow uncontrollably and form a mass called a tumor. There are treatments for cancer including chemotherapy, radiotherapy, and chemically derived drugs. For many years, herbal medicines have been used and are still used in many parts of the world as a primary source of treatment. Plants are one of the biggest boons to mankind in treating this type of disease. Hence, there are many investigations and research work is being conducted throughout the world in identifying and extracting anticancer properties from plants. Plants possessing compounds such as polyphenols, brassinosteroids, and taxols are considered anticancer metabolites [5].

The National Cancer Institute has screened approximately 35,000 plant species for potential anticancer activities. Among them, about 3000 plant species have demonstrated reproducible anticancer activity.

The aforesaid data indicate the importance of plants in primary healthcare. Keeping these aspects in view, *Curcuma karnatakensis*, an endemic species of western Ghats, is selected for the present study. The species of *Curcuma* have good medicinal value and they have been used for more than 4000 years in Ayurveda and traditional Chinese medicine. The genus *Curcuma* has been used in traditional medicine as a household remedy for many diseases, including biliary disorders, anorexia, cough, diabetic, wounds, hepatic disorders, rheumatism, and sinusitis.

The species belongs to the genus also show anticancer activity, antimicrobial, antitumor activity, antibacterial activity, antitubercular activity, toxicity activity, and wound healing activity.

METHODS

Cell lines selected for assay

Human breast cancer cells (MDA-MB-468).

Plant sample preparation

Rhizomatous root stock of both samples (Fig. 1) was washed with water and dried at 40°C. The samples were homogenized using 90% methanol and centrifuged at 6000 rpm for 10 min. The supernatant was collected and subjected for drying at 40°C and used for the anticancer study by sulforhodamine-B (SRB) assay.

Method followed

SRB assay [6].

SRB assay

Human breast cancer cells (MDA-MB-468) were used for assessing the anticancer activity of Sample A and Sample B by SRB assay at 24 h and 48 h. Wherein, a fixed number of MDA-MB-468 (10,000 cells/well) was seeded in each well of 96-well plate and kept in CO_2 incubator for

Table 1: Anticancer studies in *Curcuma karnatakensis* on human breast cancer cells (MDA-MB-468) by SRB assay after 24 h treatment

Conc.	Sample A					DADS	Sample B				
	0.1875 mg	0.375 mg	0.75 mg	1.5 mg	3 mg	1000 µM	0.1875 mg	0.375 mg	0.75 mg	1.5 mg	3 mg
% of inhibition	−21.72763	−20.4936	−18.29	0.837373	10.75364	36.25	−14.1472	−12.8691	−12.5606	−10.7977	−14.8083

SRB: Sulforhodamine-B

Table 2: Anticancer work of *Curcuma karnatakensis* on human breast cancer cells (MDA-MB-468) by SRB assay after 48 h treatment

Conc.	Sample A					DADS	Sample B				
	0.1875 mg	0.375 mg	0.75 mg	1.5 mg	3 mg	1000 µM	0.1875 mg	0.375 mg	0.75 mg	1.5 mg	3 mg
% of inhibition	18.8696	−29.7174	−18.8241	0.227894	10.20966	46	−30.629	−30.4011	−31.6317	−21.7867	−23.1085

SRB: Sulforhodamine-B

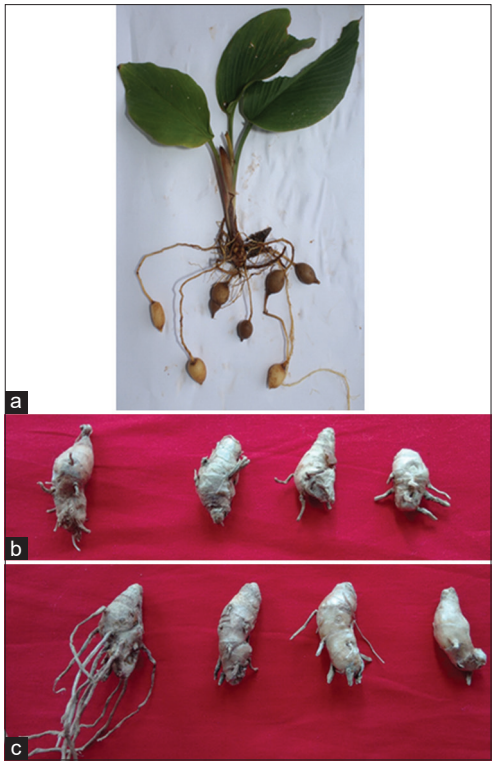

Fig. 1: *Curcuma karnatakensis* (a) plant with young rhizomatous rootstock (b) rhizomatous rootstock Sample A (c) rhizomatous rootstock Sample B

adherence of the cells for 24 h. After adherence, cells were treated with various concentrations of Samples A and B to derive the dose response curve for individual treatments for 24 h and 48 h. The optical density was read at 540 nm using a microplate reader and percentage inhibition of cancer cell growth by Sample A and B was calculated using the formula.

$$\%\ of\ inhibition = \frac{A\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

RESULTS AND DISCUSSION

Anticancer activity of Sample A at 24 h treatment is 5% for 1.5 mg and 10–20% for 3 mg (Table 1), (Fig. 2) and at 48 h treatment, Sample A shows 1–5% for 1.5 mg and 8–10% for 3 mg (Table 2), (Fig. 3). Sample B has not shown anticancer activity at both the treatment.

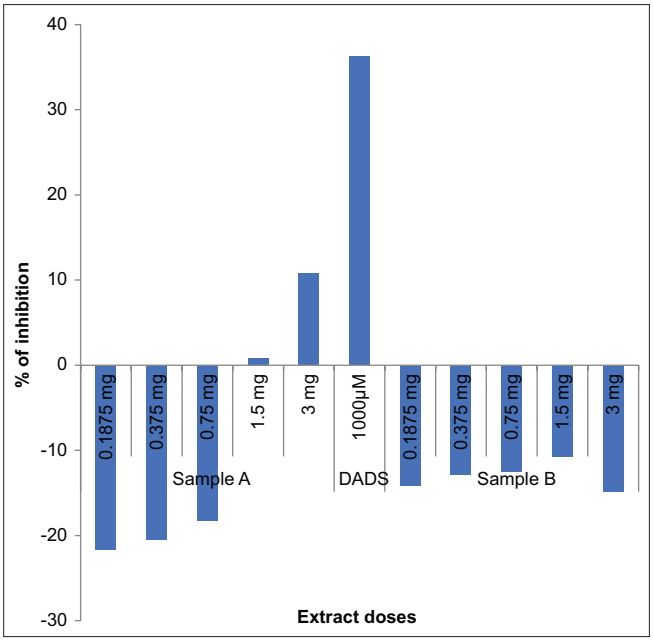

Fig. 2: Effect of extracts on MDA MB 468 at 24 h treatment

Cancer is one of the major causes for death across the world. Cancer begins when genes which normally control cell division, growth, and repair are damaged through mutation. These genes can cause cells to grow and divide uncontrollably, destroying neighboring healthy cells. Mutation can arise by chance errors in DNA replication and genes can also be damaged by carcinogens like tobacco, chemicals like acrylamide, ultraviolet light from sun. Certain viruses can also trigger gene mutation like human papilloma virus that causes cervical cancer [7] The loss of apoptotic nature by cells in their metabolic pathway leads to cancer. Cigarette smoking, alcohol intake, poor diet, and obesity can also cause cancer [8]. The cancer cells are born due to imbalance in the body and by correcting this imbalance, the cancer can be treated. Cancer disease can be prevented by adapting the dietary factors.

Plant produces the natural products which can be used either alone or in combination with other products and this can be used as anticancer drugs. Plants have a long history of use in the treatment of cancer. More than 3000 plant species have been used in the treatment of cancer [9].

The search for agents from plant sources started in 1950s with the discovery and development of the *Vinca* alkaloids, vincristine, and vinblastine from *C. roseus*, taxotere, and taxol from species of *Taxus*, campothecin analogues like topotecan from *Camptotheca acuminata*

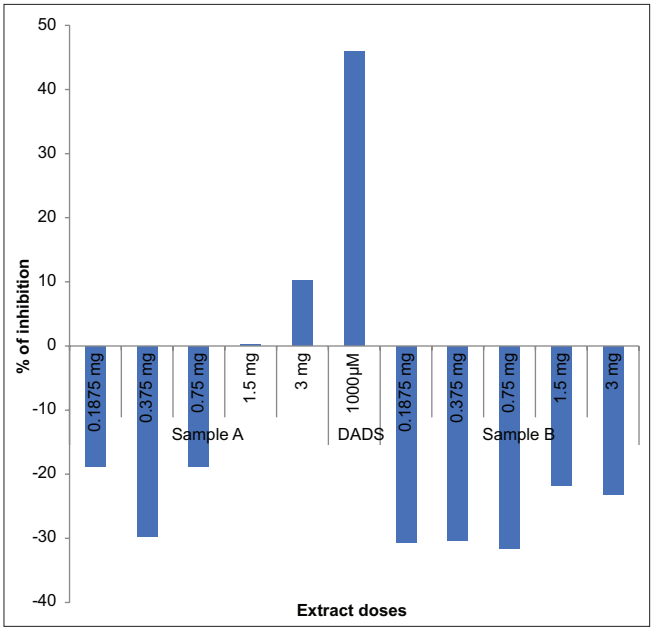

Fig. 3: Effect of extracts on MDA MB 468 at 48 h treatment

and etoposide from *Podophyllum* species. These are some of the basic components which can inhibit and prevent cancer cell proliferation. Apart from these, there are several plants which produce anti-cancerous compounds. Among them, several *Curcuma* species are reported to have anti-cancerous effects that are attributed to the presence of curcuminoids.

The high anticancer effect of Dioscorea membranacea, Dioscorea birmanica, and Siphonodon celastrineus against three human cancer cell lines by following SRB assay [10]. The aqueous extract of Curcuma zedoaria and Gloriosa superba exhibit higher activity of anticancer growth potential [11]. Curcuma caesia and Curcuma longa have shown anticancer activity at 100 µg/mL concentration [12]. The Cassia fistula, Terminalia arjuna, Cissus quadrangularis, Eclipta alba, and Gymnema sylvestre possess compounds which are anti-cancerous. As some plants availability are less and occurrence is rear, huge quantity of extraction is difficult and hence low quantities have to be tested for their properties.

In such cases, SRB assay method can be used to analysis the anticancer properties of the extraction and similar anticancer activity by SRB assay on methanolic extract of *Limnophila repens* and *Argyreia cymosa* [13] has been carried out in the past.

CONCLUSION

The anticancer activity was found to be exhibited more by Sample A than Sample B against human breast cancer cell. As the taxon selected for the present study is new to the medical and pharmaceutical field, its anticancer activity on other cancer cells has to be carried out to ascertain its anticancer activity.

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