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ANTITUMOR POTENTIAL OF *SEMECARPUS ANACARDIUM* AGAINST *EHRLICH ASCITES* CARCINOMA IN NUDE MICE

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ABSTRACT

Semecarpus anacardium (SA) popularly known as marking nut has been used in folklore for the treatment of a wide range of diseases. Extracts (hydro-alcoholic and oil) of *SA* were evaluated for their anticancer activity against *Ehrlich ascites* carcinoma (EAC) in nude mice. Extracts and standard drug (cyclophosphamide) at a dose of 20 mg/kg body weight were administered orally and continued for 10 consecutive days. The anticancer activity of *SA* was examined by determining the tumor area, tumor volume and tumor histology in experimental animal models. Both these extracts showed remarkable results in controlling the tumor in EAC bearing nude mice compared to the standard drug cyclophosphamide. Thus, the present study revealed that SA showed anticancer activity in the tested animal models.

Keywords: Semecarpus anacardium, antitumour activity, Ehrlich ascites carcinoma cells, nude mice.

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INTRODUCTION

Cancer is a major public health problem in both developed and developing countries. It was estimated that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million persons living with cancer around the world in 2002. (1, 2) Drugs from medicinal plants have played important roles in the last five decades in the treatment of cancer and most new clinical applications of plant secondary metabolites and their derivatives have been applied towards combating cancer. (3, 4) A variety of bioactive compounds and their derivatives has been shown to inhibit carcinogenesis in a number of experimental models involving initiation, promotion and progression of the tumor. Plants contain abundant quantities of these substances and have consistently been shown to be associated with a lower risk of cancers at almost every site. Efforts, therefore, are being made to identify naturally occurring anticancer compounds which would prevent or reverse the process of cancer induction and its subsequent development. (5)

Semecarpus anacardium (SA) linn (*Family: Anacardiaceae*) is distributed in sub-Himalayan region, tropical and central parts of India. The nut is commonly known as 'marking nut' and in the vernacular as 'Ballataka' or 'Bhilwa'. It has high potency and applicability in indigenous system of medicine Chemical and phytochemical analyses of *SA* nut reveal the presence of biflavonoids, phenolic compounds, bhilawanols, minerals, vitamins and amino acids. A variety of nut extract preparations from this source are used as effective against many diseases viz- arthritis, neuroprotective, infections etc (6). In this article, we describe anticancer activity of hydro-alcohol and oil extracts of *Semecarpus anacardium* against *Ehrlich ascites* carcinoma induced in nude mice.

MATERIALS AND METHODS

Plant material

Nuts of *Semecarpus anacardium* L. were obtained from the green pharmacy, Pune. The plant was authenticated at the Department of Botany, Late Karmveer Dr. P. P. Ghogrey Science College, Dhule (Maharashtra) and a voucher specimen was deposited in the Herbarium for reference purpose.

Extraction of the plant material

Cotyledons and nut shell of *Semecarpus anacardium* L. were separately extracted in hydro-alcoholic (70:30) and coconut oil as solvent by soxhelation. Extracts were tested for their preliminary phytochemical investigation along with their structural identification by FTIR spectroscopy (Fig 1, 2).

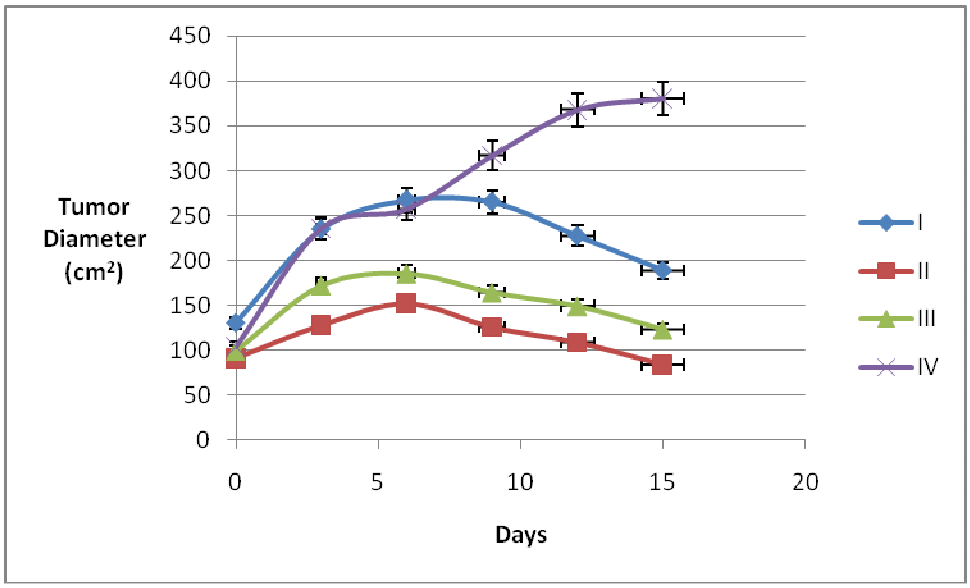

Figure
Tumor diameter measurement on various days of treatment

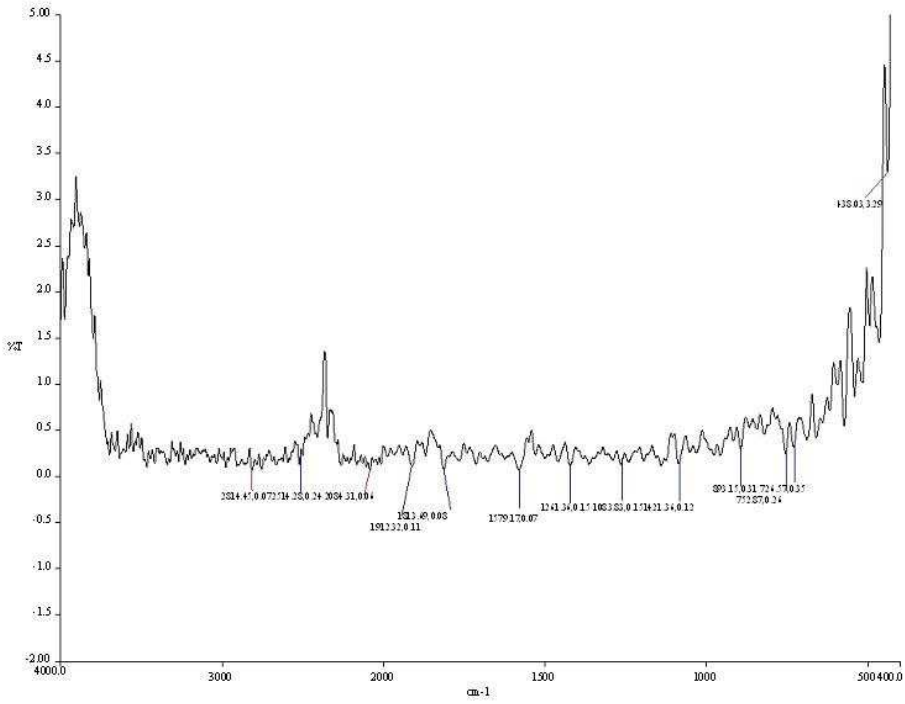

Figure 1
FTIR spectrum of hydro-alcoholic extract of SA

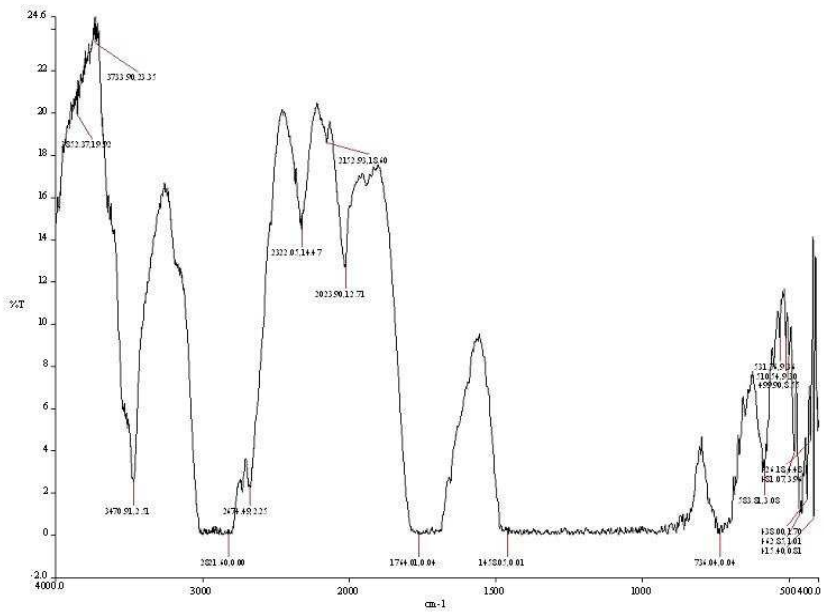

Figure 2
FTIR spectrum of oil extract of SA

Animals

Studies were carried out using nude mice of about 8 weeks of age with an average body weight of 20-25g; (Table 1) Study was conducted at National Toxicology Centre, Pune, India. The animal were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions (temperature 30^0 C) with dark and light cycle (12/12h). They were fed sterilized standard pellet diet and were given fresh water *ad libitum*. The mice were acclimatized to laboratory condition for 10 days before commencement of the experiment. All procedures described were reviewed and approved by the Animals Ethical Committee.

Table 1
Body weight of the animals during study

Sr. No.	Group	Average Body weight with S.D.
1.	Group I (Oil Extract Treated)	25.48334 ± 0.49262
2.	Group II (Hydro-alcoholic extract treated)	21.97433 ± 1.597798
3.	Group III (Cyclophosphamide treated)	21.33367 ± 1.574766
4.	Group IV (Control)	23.57 ± 1.511322

Tumor cells

A tumor cell used for anticancer activity is EAC (Ehrlich Ascites Carcinoma) cells originated from human breast carcinoma. It is an undifferentiated tumor, which has lost its epithelial character. Ehrlich ascites carcinoma (EAC) cells were obtained from National Centre for Cell Science (NCCS), Pune, India. The medium for the culture of cell line used was MEM (minimum essential medium) with 10% fetal calf serum (FCS). The (EAC) cells were injected *in vivo* in nude mice by subcutaneous inoculation of ~2 $\times 10^6$ cells/ mouse after 10 days. EAC cells of 9 days old were used for the screening of the samples.

Detection of Tumor

The animals were inspected daily through day 15 for the presence of visible and palpable tumors. The autopsies were performed in all animals from each group in order to assess and confirm tumor growth.

Experimental procedure

Four groups of nude mice of 8 weeks old with an average body weight of 20 to 24 g are used. Solid tumors were induced in all animals by injecting EAC (*Ehrlich Ascites Carcinoma*) cells subcutaneously. One group was kept as control (Group IV), while other groups were divided as oil extract treated (200 mg/kg; body weight/day) (Group I), hydro-alcoholic extract treated (200 mg/kg; body weight/day) (Group II) and standard drug cyclophosphamide (200 mg/kg; body weight/day) were administered in Group III for the treatment period of 15 days. Tumors were measured on various days, in terms of volume, weight, colour and macroscopic hemorrhages.

RESULTS

Oral administration of the extracts of *Semecarpus anacardium* nut at the dose of 20 mg\kg body weight decreased tumor area and volume of the tumor bearing mice, when compared to that of EAC control mice (Table 2). The tumor histology shows comparable changes in tumor necrosis with inflammation and comparable with standard drug (Fig.3, 4, 5). Both extracts also restored the hematological parameters. There was improvement in hematological parameters compared to that of EAC control. The Lymphocytes were increased with decreased level of Neutrophils (Table 3)

Table 2
Measurement of Tumor diameter on various days

Group	Tumor diameter					
	Day					
	0	3	6	9	12	15
I	130.64	234.755	267.43	265.13	227.295	188.955
II	91.227	128.18	152.252	125.6	108.622	83.764
III	99.796	172.406	185.724	164.72	149.518	123.502
IV	104.0667	235.7567	257.49	316.85	367.883	380.53

Table 3
Tumor measurement of the animals during study

Sr. No.	Group	Tumor weight	Tumor Volume	Remarks
1.	Group I (Oil Extract Treated)	1.4846 ± 0.706611	2.2 ± 0.706611	No skin ulceration. Geographical areas of tumour necrosis without inflammation. Areas of tumour necrosis approx. 60%.
2.	Group II (Hydro-alcoholic extract treated)	1.6622 ± 0.622664	2.2 ± 1.095445	Areas of tumour necrosis approx. 40%. Infiltrated by inflammatory cells and macrophages.
3.	Group III (Cyclophosphamide treated)	1.994667 ± 0.971808	3 ± 1	Areas of tumour necrosis approx. 60%. With bizzare tumour cells and tumour giant cells.
4.	Group IV (Control)	3.919 ± 0.998435	4 ± 1.414214	Tumour with surface ulceration and infiltration of epidermis by the tumour. Tumour is composed of spindle shaped cells with inter woven bundles. Tumour shows frequent abnormal mitosis, bizzare tumour cells and tumour giant cells. Tumour shows wide areas of necrosis (approx. 50%) which are densely infiltrated by polymorphs.

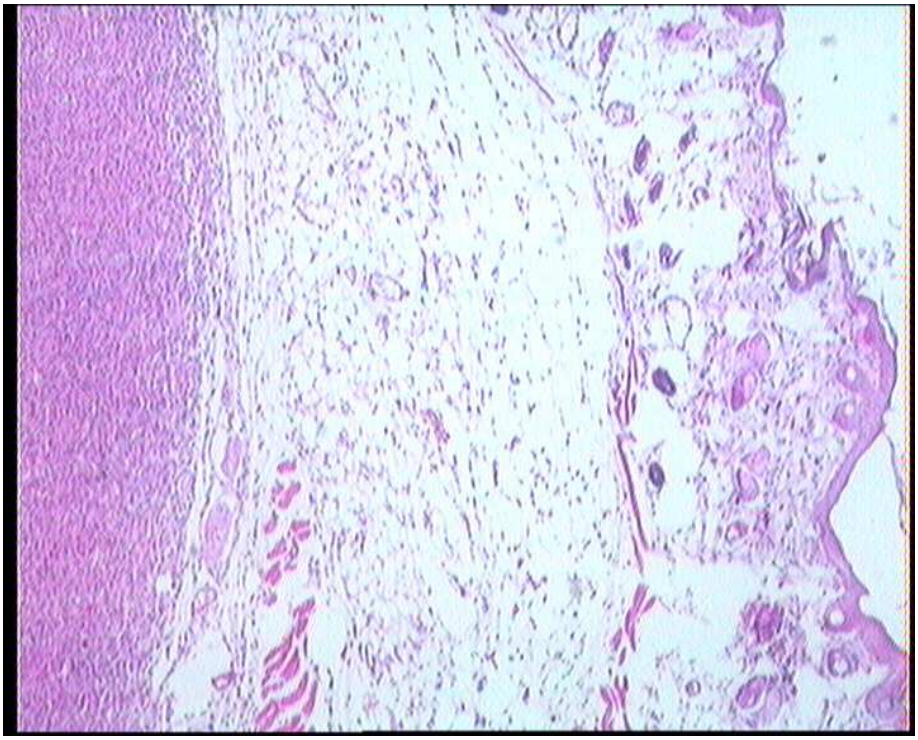

Figure 3
Tumor histology of oil extract treated animal

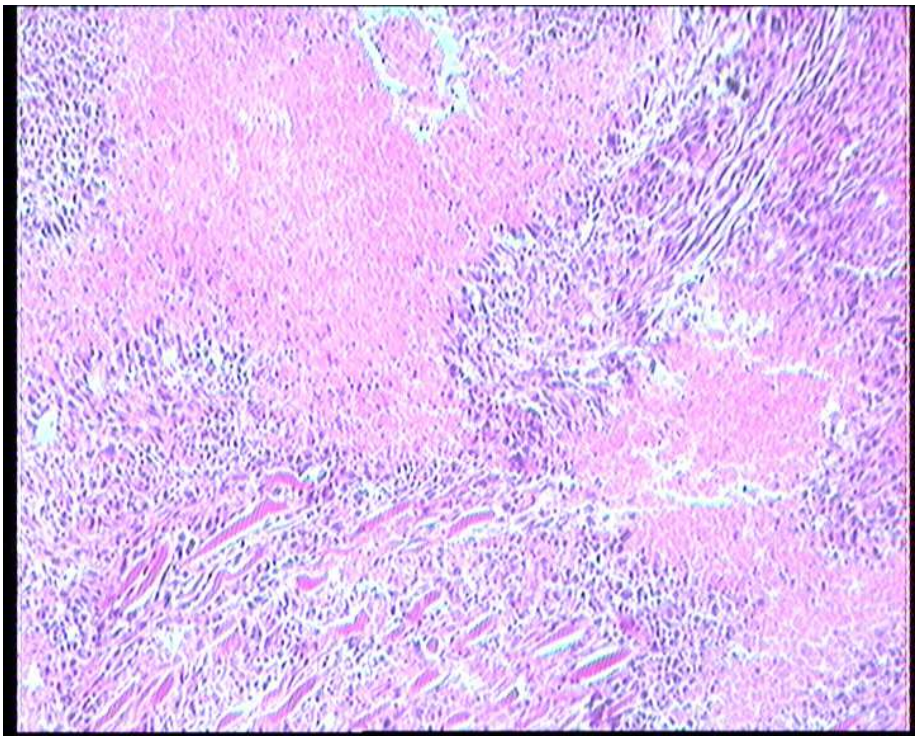

Figure 4
Tumor histology of hydro-alcoholic extract treated animal

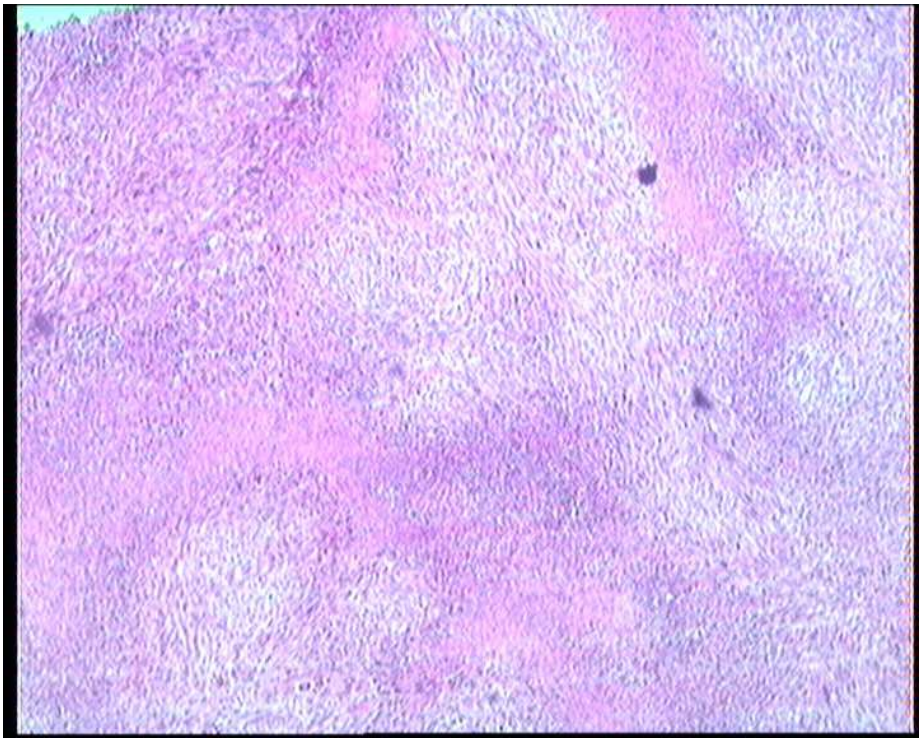

Figure 5
Tumor histology of cyclophosphamide treated animal

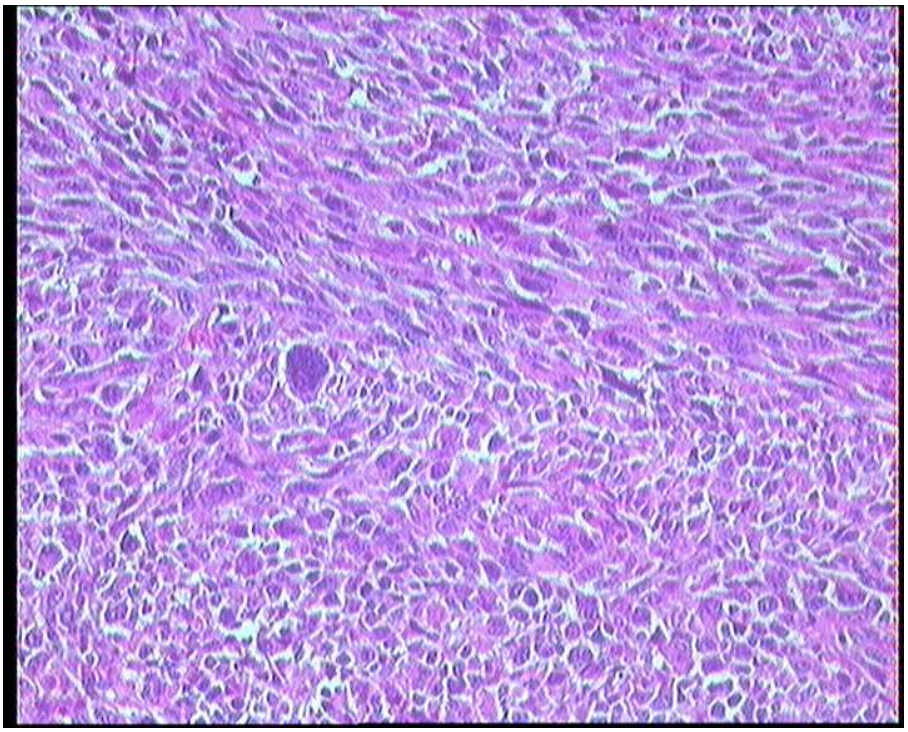

Figure 6
Tumor histology of control animal

DISCUSSION

Cancer is one of the most widespread disease in human and there is considerable scientific and commercial interest in the continuing discovery of new anticancer agents from natural product sources. Currently, over 50% of drugs used in clinical trials for anticancer activity were isolated from natural sources or are related to them. A number of active compounds have been shown to possess anticancer activity; these include flavonoids, diterpenoids, triterpenoids, and alkaloids. Several mechanisms have been proposed to explain the cancer-preventive effects of plants. These include inhibition of mutagenesis by inhibiting the metabolism, inhibition of DNA adduct formation, free-radical scavenging, and effects on cell proliferation and tumor growth. In the present study, the hydro-alcoholic and oil extracts of *Semecarpus anacrdium* nut showed inhibitory activity on *Ehrlich Ascites Carcinoma* cancer cell lines with oil extract having the greatest activity. It could therefore be concluded that the hydro-alcoholic and oil extracts of *Semecarpus anacrdium* possess anticancer activities. Bioflavonoids have been implicated as responsible for the anticancer activities of some medicinal plants. Literature showed presence of the flavonoids in the nut like biflavones A, C, A_1, A_2, tetrahydrorobustaflavone, B (tetrahydromentoflavone) (7), jeediflavone, (8,9), semecarpuflavone (10) and gulluflavone (11). Oil from nuts, bhilavinol, contains a mixture of phenolic compounds mainly of 1,2-dihydroxy-3 (pentadecadienyl-8, 11) benzene and 1,2-dihydroxy-3 (pentadecadienyl-8', 11')- benzene (12). Thus, flavonoids may be responsible for the anticancer activity of *Semecarpus anacrdium*. Further research is needed to unravel the specific bioactive compounds responsible for the anticancer activity of the extracts of *Semecarpus anacrdium*. In conclusion, the study has not only established the anticancer property of the extracts of *Semecarpus anacrdium* but also its immunomodulatory activity.

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