EVALUATION OF *INVIVO* ACTIVITY OF *MARTYNIA ANNUA* AND *CORCHORUS TRILOCULARIS* PLANTS EXTRACT FOR ANTICANCER ACTIVITY

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ABSTRACT

The present work evaluates the anticancer, antioxidant, properties of diverse medicinal plants frequently used in Indian traditional medication. The selected *Martynia annua*, and *Corchorus trilocularis* extracted in different solvents were evaluated for their *in vivo* anticancer activities. The results obtained indicate that *Martynia annua*, and *Corchorus trilocularis* has potent cytotoxic activity toward the selected cancer cells such as A459, H522, H460 cells. The results of the antioxidant study revealed that the selected plants were found to be effective 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl (OH), Nitric oxide, ABTS and hydroxyl radical scavenging agents. High-performance thin layer chromatography (HPTLC) fingerprint of flavonoids was used as a measure of quality control of the selected plant samples. The results of the present findings strengthen the potential of the selected plants as a resource for the discovery of novel anticancer, anti-inflammatory, and antioxidant agents. This study suggests that Ethanolic extracts of *Martynia annua*, and *Corchorus trilocularis* can be used for further isolation and purification of active principles.

Keywords: Anticancer, Antioxidant, Medicinal plants, Phytochemicals, In-vivo Activity.

1. INTRODUCTION

Cancer is one of the most life-threatening diseases, with more than 100 different types occurring due to some molecular changes within the cell. It is the third leading cause of death worldwide following cardiovascular and infectious diseases.[1] It is estimated that 12.5% of the population dies due to cancer (WHO, 2004). The disease is widely prevalent, and in the West, almost a third of the population develops cancer at some point of time during their life. Although the mortality due to cancer is high, many advances have been made both in terms of treatment and understanding the biology of the disease at the molecular level.[2]

Cancer is a general term for uncontrolled and abnormal growth of cells, most types form a lump or mass called tumour. Persistence of growth is a salient feature of tumour cells, and this distinguishes them from other normal healthy body cells. Not all tumours are cancer; any tumour that is not a cancer is said to be benign or less technically, simple tumour. If the process of tumour formation endures, it leads to death of the individual [3]. Viruses are the usual infectious agents that cause cancer, but bacteria and parasites may also have an effect. Malignant tumours are generally cancers and are caused by a breakdown of genetic material of resulting cells

and these may be due to the effect of physical carcinogenic agents, such as tobacco smoke, ionizing radiation, chemicals, or infectious agents [4] which damage the DNA of a critical gene in a cell; this DNA damage invokes mutations leading to irregularities in the gene function.[5]

Moreover, it is increasingly being realized that many of today's diseases are due to the "oxidative stress" that results from an imbalance between the formation and neutralization of prooxidants. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids, and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, aging, and inflammatory diseases.[6,7] All cells are exposed to oxidative stress, and thus, oxidation and free radicals may be important in carcinogenesis at multiple tumor sites.

Due to lack of effective drugs, cost of chemotherapeutic agents, and the side effects of anticancer drugs, cancer can be a cause of death. Therefore, efforts are still being made to search for effective naturally occurring anticarcinogens that would prevent, slow, or reverse cancer development. Medicinal plants have a special place in the management of cancer. It is estimated that plant-derived compounds in one or the other way constitute more than 50% of anticancer agents.[8,9] Numerous cancer research studies have been conducted using traditional medicinal plants in an effort to discover new therapeutic agents that lack the toxic side effects associated with the present chemotherapeutic agents. Taking into consideration the above facts, an attempt has been made to evaluate the anticancer, and antioxidant activities of selective medicinal plants used in Indian traditional medicine system.

2. MATERIALS AND METHODS

Materials

Leaves of *Martynia annua* and, *Corchorus trilocularis* was procured from local area of Bhopal from its pure quality. They prepared the leaves extract as 65% ethanolic extract, for which 65% succussed ethanol was used as for the related experiments.

Collection of plant material

The plants used in this study consisted of *Martynia annua* and, *Corchorus trilocularis* were collected from local area and purchased commercially from recognized Ayurvedic shop from Bhopal (Madhya Pradesh Province).

Authentication of plant material

The plant material was collected from local area of Bhopal and was authenticated at the Department of Botany, Hari Singh Gour University Sagar. Voucher specimens of the collected plants were deposited in the

herbarium center of the host institute. The shade-dried and powdered plant samples were preserved for further experiments.

Extraction

Sequential solvent extraction was carried out by following the procedure given in [10]. The powdered leaves was subjected to the percolation type of extraction with solvents of increasing polarity added sequentially. Solvents (150ml each) such as petroleum ether, chloroform, ethyl acetate, methanol and water were used.

Phytochemical tests

The phytochemical analysis was carried out using the protocol given. The five extracts of *Martynia annua, Corchorus trilocularis*, namely petroleum ether extraction, chloroform extraction, ethyl acetate extraction, methanol extraction and aqueous extraction were subjected to qualitative phytochemical tests to check the presence of alkaloids, Glycoside, tannins, saponins, triterpenoids, steroids, anthraquinones and glycosides**.**

Animals

Inbred healthy white wistar rats were reared in the animal house of the Department of Pharmacy, SRK University, Bhopal, India. Ethical clearance for the experimental set up was obtained from the Institutional Ethical Committee, University of SRK. 3 to 4 months old randomized rats, weighing between 80g and 90g, served as the materials for the present study. Animals were maintained in hygienic condition in the animal house in polypropylene cages (temperature, 24±2ºC; humidity, 55±5%, 12h light/dark cycles), allowed free drinking water and basal diet *ad libitum*, as per the guidelines of the Animal Welfare Committee, Department of Pharmacy, SRK University, Bhopal.

Ascitic tumour model/ induction of experimental tumour

Experimental tumours have great importance with regard to modelling and Ehrlich Ascites Carcinoma (EAC) is one of the commonest used. It appeared, firstly, as a spontaneous breast cancer in a female mouse and then Ehrlich and Appellant in 1905 used it as a trial tumour by transplanting tumour tissues subcutaneously from mouse to mouse. In 1932, Loewenthal and Jahn obtained the liquid form in the peritoneum of the mouse and named it as Ehrlich's ascites carcinoma (EAC). EAC is referred to as an undifferentiated carcinoma and is originally hyper-diploid, has high transplantable capability, non-regression, rapid proliferation, shorter life span, 100 % malignancy and it also does not have a tumour-specific transplantation antigen. The effusion, which contained neoplastic cells that are proliferated after injection of tumour cells into the peritoneal cavity, is referred to as ascites [11].

Preparation of test samples :

Extracts were prepared as described earlier The petroleum ether and ethanol extracts of *Martynia annua, and Corchorus trilocularis* were preserved in a closed container below 20ºC. It was freshly prepared at three different doses of 50, 100 and 200 mg/kg for both petroleum ether and ethanol extracts in water for injection every day before dosing the animals.

Induction of experimental tumour

Ehrlich Ascites Carcinoma (EAC) cells were aspirated aseptically from the tumour bearing mice using 18 G needle. The cells were counted and phosphate-buffered saline (pH 7.2) was used to adjust the volume and the cell viability was checked. Cell suspensions containing 2.5×10^{6} cells were injected intraperitoneally to obtain ascitic tumour in mice.

Evaluation of tumour growth response:

The antitumour potential of extracts was assessed by following changes in body weight, determination of total ascitic fluid volume, packed cell volume and number of viable and non-viable cells.

- **Body weight analysis:**

  After tumour cell inoculation, all the mice were weighed on consecutive days up to 14 days [12].

- **Estimation of total ascites fluid volume:**

  Ascitic fluid of EAC tumour bearing mice was collected on the 15th day in a graduated centrifuge, and fluid volume was measured.

- **Estimation of packed cell volume:**

  Ascitic fluid was centrifuged at 2000 RPM for 20 min. Volume of cells sediment indicates the packed cell volume.

- **Viable and non-viable cell count:**

  The cells aspirated from the treated mouse was stained with Trypan blue dye (0.4% in normal saline). The cells that did not take up the dye were alive and those that took the stain were dead and were counted.

Determination of the mean survival time (MST) and percentage increase in life span (% ILS)

The survival time of EAC inoculated mouse was analysed by recording the daily mortality. Mean survival time of each group and percentage increase in life span was calculated from the given formula,

$$\text{MST} = \frac{\text{Day of first death} + \text{Day of last death}}{2}$$

$$\%\ \text{ILS} = \frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of control group}} \times 100$$

Solid tumour model

DLA is a transplantable poorly differentiated malignant tumour, which appeared originally as lymphocyte in a mouse. It grows both in solid and ascitic forms [13]. Following transplantation of Dalton's ascites cells (AC) into the peritoneal cavity of healthy mouse, tumour-genesis begins immediately and aggressively. The recipient mice survive up to 2-3 weeks [14].

Induction of experimental tumour

Extract preparation and cell line propagation were done as described in section 4.2.2 and 5.2.1. DLA cells were aspirated from peritoneal cavity of the tumour bearing mice, and 0.1 mL containing 1×10^{6} cells were injected subcutaneously (*sc)* . into the right hind limb of *Swiss albino* mice. five groups were selected, of which each group contains six animals. All groups were treated with DLA (1×10^6 cells) on day 0. Group 1 acted as normal control, Group II received normal saline (0.9%) and Group III served as standard drug, Group IV, V, received different doses of MA & CT. The treatments were done on successive 30 days.

Effects of MA and CT on solid tumour development:

The radii of developing tumours were measured using vernier callipers on every 3rd day and tumour volume was calculated using the formula

$$V = 4/3\pi r1^2 r2$$

Where, r_1 and r_2 are the radius of the tumours at two different directions

Effect of MA and CT on percentage inhibition of tumour growth:

On 30th day all the mice were deprived of food overnight and euthanized by cervical dislocation. Tumours were excised, 24 h after the last treatment, washed with PBS and weighed. Tumour weight of treated and its untreated counterparts were recorded and compared. And the percentage of inhibition of tumour volume in animals was calculated

3. RESULTS AND DISCUSSION

Ascites tumour model

Effect on tumour growth response

Body weight: Tumour development was noticed from day 6 and there was a gradual rise in body weight due to accumulation of ascites fluid in the peritoneum of EAC mice, especially in the positive control group (45.54±66), whereas a significant reduction of gain in body weight was found in the standard (cisplatin) treated groups (7.12±09). On comparing the extract treated groups, all groups exhibited significant differences in a dose-dependent manner and MA 400 mg/kg showed a maximum decrease in body-weight (19.38±1.9) (Table.1).

Both extract groups showed significant differences of gain in body weight when compared with the positive control group indicating the effectiveness of the test extracts. Cisplatin (Standard) treated group caused 91% decrease in body weight, while group IV and V MA (400mg/kg) and CT (200mg/kg) registered only 60.95 and 54.24% respectively.

Effect on mean survival time (MST) and percentage increase in life span (% ILS)

Mean survival time and percentage increase in life span are two important parameters used for screening of any anticancer agent. A compound can be considered as cytotoxic if it exhibits more than 25% increase in percentage life span. A significant drop in MST was observed in EAC induced mice (14 days) when compared with standard mice (33 days). In the control group, on day 15 the first death was reported, and all mice were

dead by day 18. However, cisplatin caused a significant increase in the MST (33 days) and % ILS (115) when compared to the vehicle group. Both petroleum and ethanol extracts significantly improved the MST and all groups exhibited a dose-dependent increase in the life span. Among the six treatments, the petroleum ether extract of MA 400 mg/kg was found to be the most effective percentage inhibition of 81.8% (Table. 2, Fig. 1).

Table. 1. Effect of petroleum ether and ethanol extracts of *Martynia annua and Corchorus trilocularis*on tumour growth response of EAC inoculated *Swiss albino mice*.

Group	Tumour weight (g)	Tumour volume (mL)	Packed cell volume (mL)
Control	-	-	0.33 ± 0.02^{x}
Positive Control	9.96 ± 0.1180^{a}	8.875 ± 0.2045^{a}	5.867 ± 0.275^{a}
Standard	2.645 ± 0.0234^{x}	0.9878 ± 0.0174^{x}	0.4856 ± 0.1787^{x}
MA 400mg	$3.675\pm0.1080^{x,a}$	$1.783\pm0.0667^{x,c}$	0.9687 ± 0.0589^{x}
CT3 200mg	$4.745\pm0.1479^{x,a}$	$3.784\pm0.1877^{x,a}$	$1.937\pm0.8767^{x,a}$

n=6, values are expressed as mean ± SEM. Statistica l analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. ^{x} $p<0.001$, $^{y}p<0.01$ and $^{z}p<0.05$ as compared to control, ^{a} $p<0.001$ ^{b} $p<0.01$ and ^{c} $p<0.05$ as compared to standard, ns- non significant.

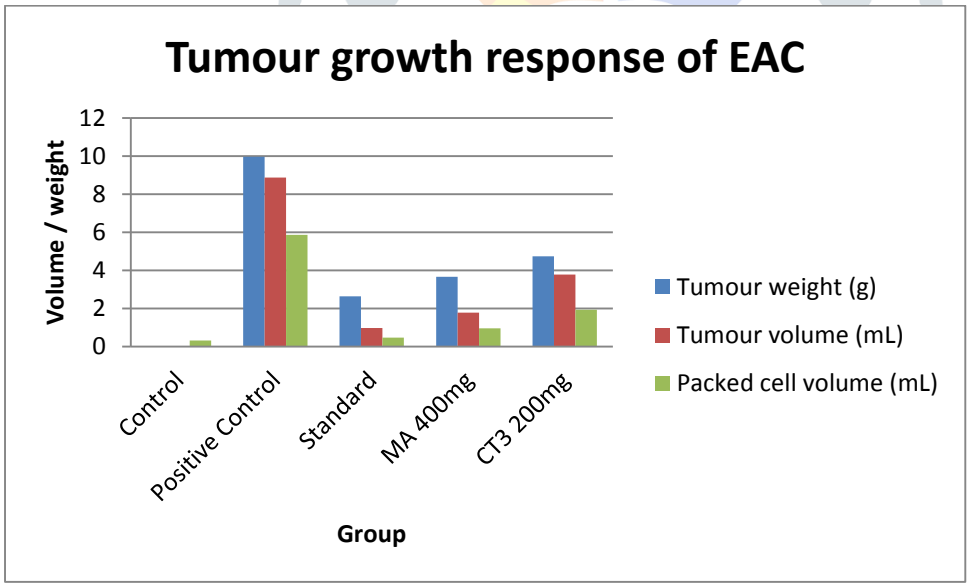

Fig. 1. Tumour growth response of EAC inoculated in *Swiss albino mice*.

Table. 2. Effect of different concentrations of petroleum ether and ethanol extracts of *Martynia annua and Corchorus trilocularis*in gain in body weight, MST and % ILS.

GROUPS	% Gain in body weight (g)	% Decrease in Body weight (g)	MST (days)	% ILS
Control	2.21x,ns	-	-	-
Positive Control	45.54±.66a	-	15.5±0.53a	-
Standard	7.12±.09x	93	33±0.56x	115.25
MA400mg	19.38±.19x, a	65.85	30±1.39x, c	84.87
CT 200mg	20.12±.22x, a	57.28	24±0.48x, a	55.54

n=6, values are expressed as mean ± SEM. Statistica l analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. ^{x}p <0.001, ^{y}p<0.01and ^{z}p<0.05 as compared to control, ^{a} p<0.001, ^{b} p<0.01 and ^{c} p<0.05 as compared to standard, ns – non significant.

Solid tumour model

Effect on mean survival time (MST) and percentage increase in life span (% ILS)

Mean survival time and percentage increase in life span are two important parameters used for screening of any anticancer agent. A compound can be considered as cytotoxic if it exhibits more than 25% increase in percentage life span. A significant drop in MST was observed in EAC induced mice (15.6 days) when compared with standard mice (20 days). In the control group, on day 15 the first death was reported, and all mice were dead by day 19. However, cisplatin caused a significant increase in the MST (22 days) and % ILS (58) when compared to the vehicle group. Both petroleum and ethanol extracts significantly improved the MST and all groups exhibited a dose-dependent increase in the life span. Among the six treatments, the petroleum ether extract of MA 400 mg/kg was found to be the most effective showing a percentage inhibition of 48.8% and survival time is (21 days) (Table.3, Fig. 2)

Table.3. Effect on mean survival time (MST) and percentage increase in life span (% ILS) on solid tumour study of *Martania annua and Corchorus trilocularis*

GROUP OF ANIMALS	MEAN SURVIVAL DAYS	INCREASE IN LIFE SPAN (%)
Normal	15.67±3.63	-
Positive Control	20.40±4.41	22.34
Standard	22.17±3.62	58.87
MA 400mg	21.33±4.38	48.89
CT 200mg	20.36±1.50	45.20

n=6, values are expressed as mean ± SEM. Statistica l analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. [x] p <0.001, [y]p<0.01 and [z]p<0.05 as compared to control, [a] p<0.001 [b] p<0.01 and [c] p<0.05 as compared to standard, ns- non significant.

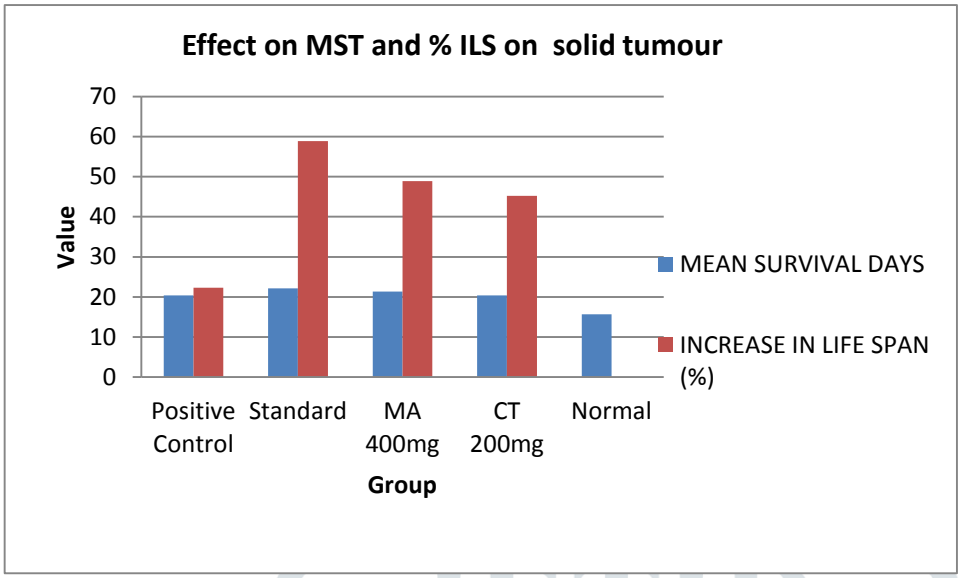

Fig. 2. Effect on mean survival time (MST) and percentage increase in life span (% ILS) on solid tumour study of *Martania annua and Corchorus trilocularis*

Percentage inhibition of tumour weight and tumour volume.

To confirm these findings, the tumour weight after excision was also recorded. A significant reduction in average tumour weight was found in the treated animals compared to the control animals. There was a reduction in tumour weight of *Martynia annua* 400 mg/kg *and Corchorus trilocularis* 200 mg/kg treated animals (77.54% and 75.42%) and was comparable with that of standard cisplatin (95%). (Table.4)

Table.4. Effect of *Martynia annua and Corchorus trilocularis* on percentage inhibition of tumour weight and tumour volume of DLA induced *Swiss albino mice*.

Group	Average tumour weight ±SEM (g)	% Reduction of tumour volume after 30th day	Percentage inhibition
Positive control	0.3020±0.01020	3.21±0.02	00.00
Standard	0.02020±0.00090	1.09±0.06	95.074
MA 400mg	0.2250±0.01300	1.88±0.03	77.544
CT 200mg	0.2100±0.01010	1.26±0.04	75.426

n=6, values are expressed as mean ± SEM. Statistica l analyses were done by one-way ANOVA followed by Tukey's multiple comparison tests. [x] p <0.001, [y]p<0.01 and [z]p<0.05 as compared to control, [a] p<0.001 [b] p<0.01 and [c] p<0.05 as compared to standard, ns- non significant.

Statistical Analysis

The values obtained with the test samples were compared with those of standard and control at the different confidence level by Graph Pad Prism (version 5.0) software. Analysis was done by one-way ANOVA followed by Tukey's multiple comparison test. ^{x} $p<0.001$, $^{y}p<0.01$, $^{z}p<0.05$ as compared to control, ^{a} $p<0.001$ ^{b} $p<0.01$ ^{c} $p<0.05$ as compared to standard. Values are expressed as mean ± SEM.

4. CONCLUSION

It can be summarized that the plants selected in the present study having importance in traditional medicine can be considered as a source for the isolation, identification, and development of novel and effective anticancer, and antioxidant agents. Nevertheless, the research data of the present findings may serve as a guideline for the standardization and validation of natural drugs containing the selected medicinal plants as ingredients.

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