IN VITRO ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *MARTYNIA ANNUA* AND*CORCHORUS TRILOCULARIS* PLANTS EXTRACT FOR ANTICANCER ACTIVITY

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Abstract

The present study was carried out to evaluate 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 vitro* anticancer and antioxidant 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, Invitro Activity

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.

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.

Reagents

Dulbecco’s Modified Eagle Medium (DMEM), Roswell Park Memorial Institute-1640(RPMI-1640), fetal bovine serum (FBS) and 0.05% trypsin-EDTA were purchased from HiMedia Laboratories Pvt. Ltd.Mumbai. Antibiotic antimicotic solution was purchased from Himedia, India. All organic solvents used were of 99%

HPLC grade. Acrydine orange (AO), 3-(4, 5-Dimethyl-thiazol-2-yl)-2, S-diphenyltetrazolium bromide (MTT), ethidium bromide (EB), Rhodamine 123, 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI), Hoechst 33258, dichloro-dihydrofluorescein diacetate (H_2DCFDA), Rhodamine 123 and all other chemicals were purchased from Sigma Chemical ltd. All chemicals used were of analytical grade. Cell culture plastic wares were obtained from Indiamart. Annexin V-FITC, monoclonal antibodies against Bax, Bcl_2, cytochrome-c, caspase-3, p53, apaf-1, PARP and GAPDH were purchased from Chromous Biotech, India. All the primers were procured from Bio-serve Biotechnologies India Pvt. Ltd. Mumbai mulv reverse transcriptase, Taq DNA polymerase, dNTPs and other RT-PCR reagents were purchased from Chromous Biotech, India.

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 [10-11]. 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**.**

Cell Lines Culture

A549, NCI-H522 (H522) and NCI-H460 (H460) human NSCLC cell lines were procured from National Centre for Cell Science (NCCS), Pune, India.A549 cells were cultured in DMEM and H522, H460 cells were cultured in RPMI-1640, supplemented with 10% heat inactivated FBS and 1% antibiotic antimicotic solution maintained at 37ºC with 5% CO_2 in a humidified incubator. The cells were harvested with 0.05% Trypsin and 0.52mM EDTA in PBS, plated at required cell numbers and were allowed to adhere for 24h before treatment.PBMC

were cultured in RPMI-1640 media supplemented with 5% FBS and 1% antibiotic antimicotic solution and maintained at 37°C in a humidified incubator with 5% CO_2[12-13].

Determination of cell viability by MTT assay

This is a colorimetric assay that measures the reduction of yellow coloured MTT by mitochondrial succinate dehydrogenase. The MTT enters into the cells and passes to the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells.

Cell viability was assessed using MTT followed by the method. NSCLC cells ($1x10^5$cells/ml) were cultured for 24h on 96-well micro plates. The cells were incubated for different time-points for different modes of studies with and without drugs.[14]

Materials for MTT

Freshly prepared MTT (5mg/ml in PBS) was filtered and kept in dark, at 4ºC.

Acidic isopropanol (0.1N HCl in absolute isopropanol) 96 flat bottom well plate.

Antioxidant Study

DPPH

DPPH radical scavenging assay was carried out as per the method reported earlier, with slight modifications.[15,16] Briefly, 1 ml of the test solution (individual plant extracts) was added to an equal quantity of 0.1 mM solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mM) was used as the reference compound.

Hydroxyl (OH) radical scavenging assay

The OH radical scavenging activity was determined using Fenton reaction.[17] The reaction mixture contained 60 µl of $FeCl_2$ (1 mM), 90 µl of 1,10-phenanthroline (1 mM), 2.4 ml of phosphate buffer (0.2 M, pH 7.8), 150 µl of H_2O_2 (0.17 M), and 1.5 ml of individual plant extracts (1 mg/ml). The reaction was started by adding H_2O_2. After 5 min incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1 mM) was used as the reference compound.

Nitric Oxide

Methanolic extract of *Martynia annua, Corchorus trilocularis* showed moderately good nitric oxide scavenging activity between 50 and 250µg/ml. At a concentration of 250 µg, the scavenging activity of *Martynia annua*, and*Corchorus trilocularis* was 81.4 %.The percentages of inhibitions wereincreased with increasing concentration of the extracts. In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological condition. The Figure 6 shows the dose-response curve of Nitric Oxide Scavenging activity of the *Martynia annua, Corchorus trilocularis.*

Results and Discussion

Preliminary Phytochemical screening

Preliminay phytochemical evaluation of *Martynia annua, Corchorus trilocularis* leaves indicatedthe presence of alkaloids,glycosides, tannins, resins, phenolics, flavanoids, steroids, carbohydrates and proteins **(Table 1).**

Preliminary Phytochemical evaluation

Sample	Alkaloids	Glycosides	Flavanoids	Phenolics	Saponins	Tannins	Carbohydrates	Terpenoids	Proteins	Steroids
Corchorus triloculariis	+++	+++	+++	+++	++	+++	++	+++	++	+
Martynia annua	+++	+++	+++	+++	++	+	++	+++	++	+

In Vitro Antitumour Evaluation by MTT assay

Antitumour evaluation of extracts

*Martynia annua and Corchorus trilocularis*ethanol extracts weresubjected to MTT assay. Different cell lines used were cultured in A459, H522, H460 cells. The results are depicted in the (**Table 2, Fig. 1)**. It is very clear from the results that *Martynia annua and Corchorus trilocularis*are showing comparative activity comparative to control treated group.

Table. 2 The IC_{50} value of extracts by MTT assay

GROUP	IC_{50} VALUE		
	CELL LINES		
	A549	H522	H460
Negative control	300	280	340
Martynia annua	32.88	38.80	42.77
Corchorus trilocularis	40.00	42.77	45.88

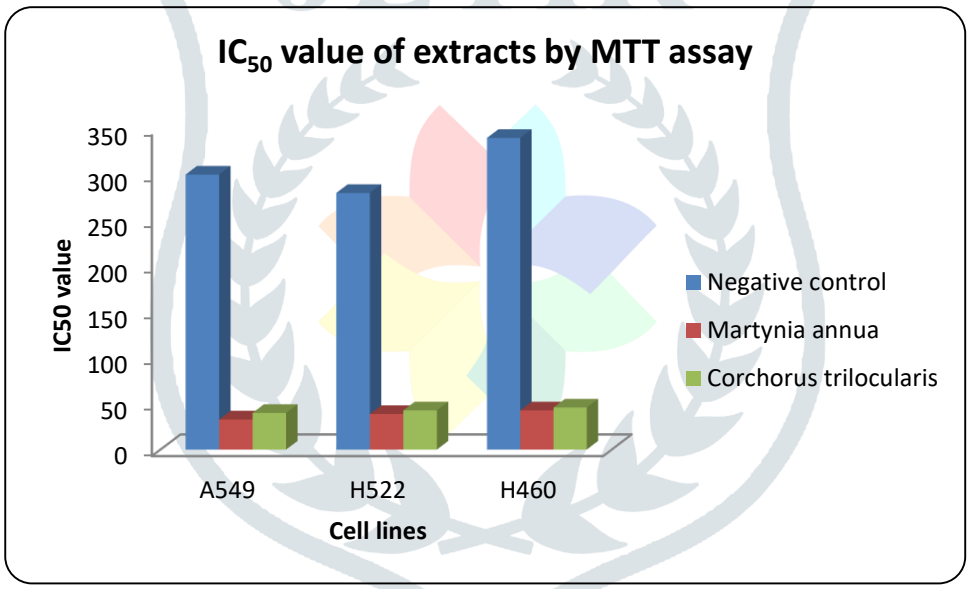

Fig. 1. The IC_{50} value of extracts by MTT assay

In Vitro Antioxidant activity

In vitro antioxidant study of the leaf extract of all theselected plants were carried out by DPPH, nitric oxide, ABTS and hydroxy radical scavenging methods. The results are shown in **Table 3- 4 and Fig. 2-3**.

Table 3. Antioxidant study of *Martynia annua*

Conc.(µg/ml)	Percentage inhibition (%)			
	DPPH	NITRIC OXIDE	ABTS	HYDROXY RADICAL

100	21.65	07.39	14.78	33.26
200	25.98	25.24	23.86	38.01
300	35.79	34.42	33.47	54.38
400	42.66	44.03	45.09	55.54
500	55.65	54.38	54.49	66.21
IC50 (µg/ml)	460	450	420	280
Ascorbic acid (IC50 (µg/ml)	12.34	32.12	15.13	22.36

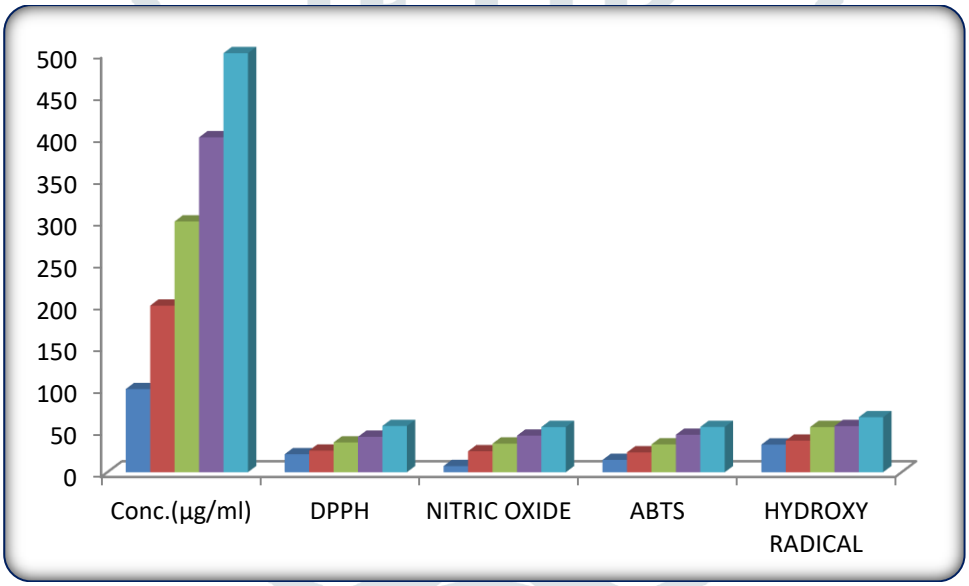

Fig 2. Antioxidant study of *Martynia annua*

Table 4. Antioxidant study of *Corchorus trilocularis* leafextract in ethanol

Con c.(µg/ml)	Percentage inhibition (%)			
	DPPH	NITRIC OXIDE	ABTS	HYDROXY RADICAL
100	45.00	47.03	42.75	48.55
200	65.55	59.65	50.25	55.38
300	60.66	61.65	55.70	66.75

400	64.67	67.99	66.35	75.00
500	75.50	76.14	70.21	79.80
IC_50 (µg/ml)	175	160	180	150
Ascorbic acid (IC_50 (µg/ml)	15.34	30.12	25.13	24.89

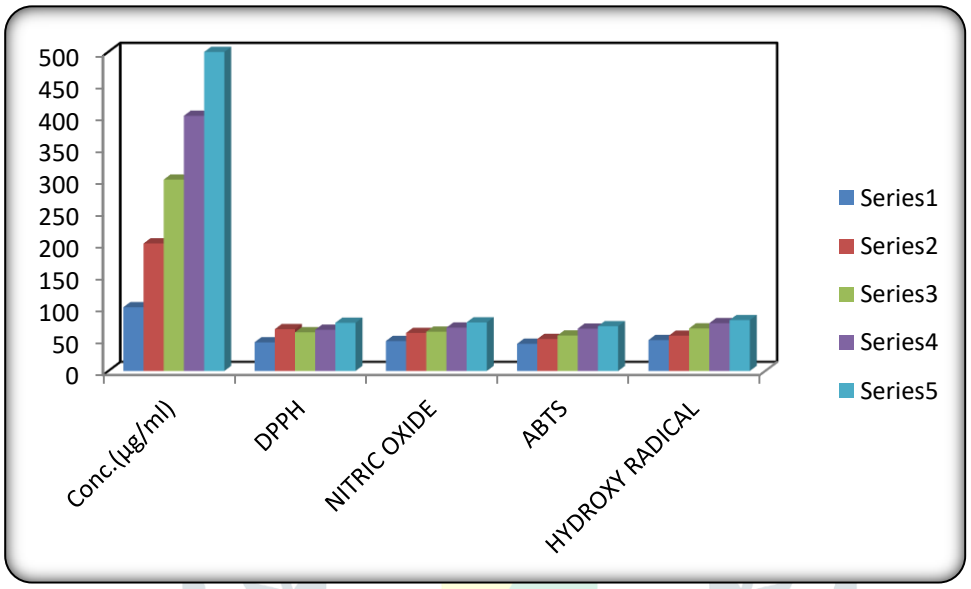

Fig 3. Antioxidant study of *Corchorus trilocularis* leafextract in ethanol

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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