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## Anticancer Properties of Phytochemicals Present in Indian Medicinal Plants.

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### ABSTRACT

Cancer is a major public health burden in both developed and developing countries. Anticancer activity is the effect of natural and synthetic or biological and chemical agents to reverse, suppress or prevent carcinogenic progression. Several synthetic agents are used to cure the disease but they have their toxicity and hence the research is going on to investigate the plant derived chemotherapeutic agents. Therefore an attempt has been made to review different *in vitro* and *in vivo* methods for estimating anticancer properties of natural products from medicinal plants. In the present study, 5 anticancer medicinal plants (*Abutilon indicum*, *Adathodavisca*, *Daturastramonium*, *Lantana camara* and *Tridaxprocumbens*) of Indian origin belonging to 5 different families are reported along with detailed information regarding part used, extract used, type of the model used (MTT method), types of tested cancer cell lines (HCT116 cells), etc. The result showed that alkaloids & flavonoids of *Lantana camara* & *Daturastramonium* have shown dose dependent activity against HCT-116 cells with IC50 values of 52.07, 43.82 & 57.23, 36.9 µg/ml respectively. In comparison to alkaloids of *Abutilon indicum* & *Tridaxprocumbens*, there is a relative potent activity in alkaloids & flavonoids of *Lantana camara* & *Datura strontium*. These plants continue to be used against various types of tumours such as sarcoma, lymphoma, carcinoma and leukemia. All these plants are potential candidates for *in vivo* studies since they are showing good *in vitro* anticancer activity.

**Keywords:** Anticancer Medicinal Plants, Indian origin, Tumors, *in vitro* and *in vivo* Methods.

### INTRODUCTION

Cancer is one of the major life-threatening and public health problems in both developing and developed countries. In 2014 the World Cancer Society reports that the leading causes of morbidity and mortality worldwide are cancer. Approximately 14 million people were reported to suffer from cancer and 8.2 million cancer related deaths in 2012. The number of new cases is expected to rise by about 70% over the next 2 decades. Among men, the 5 most common sites of cancer diagnosed in 2012 were lung, prostate, colorectum, stomach, and liver. 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 (Kelloff GJ, 2008).

Most of the women are suffering from breast cancer it's become most common. The incidence of breast cancer is the highest in Pakistan compare the South-Central Asian countries. It is the most frequent malignancy in women and accounts for 38.5% of all female cancers. About half (43.7%) of all breast cancers are detected in an advanced stage (Doll R, Peto R, 2003). Colon cancer is the second most common cause of cancer deaths in the US. Prostate cancer is the most frequently diagnosed cancer among men in the US, and ranks second to skin cancer, with an estimated 180,000 new cases and 37,000 deaths expected to occur by the American Cancer Society each year (Soliman AS, 2006).

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 (American Cancer

Society,1999).These changes contribute to cancer,today, despite considerable efforts; cancer still remains an aggressive killer worldwide. So the novel synthetic chemotherapeutic agents currently in use clinically have not succeeded in fulfilling expectations despite the considerable cost of their development (Braca A, Sortino C 2002).

Therefore there is a constant demand to develop new, effective, and affordable anticancer drugs. From the dawn of ancient medicine, chemical compounds derived from plants have been used to treat human diseases. Natural products have received increasing attention over the past 30 years for their potential as novel cancer preventive and therapeutic agents (Maxwell SR,1995). In parallel, there is increasing evidence for the potential of plant-derived compounds as inhibitors of various stages of tumor genesis and associated inflammatory processes, underlining the importance of these products in cancer prevention and therapy(Warren JS,1990).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.

## MATERIALS AND METHODS

**1.1 Plant Collection and Identification:-** The Plant materials *Adathodavisca*, *Daturastramonium*, *Lantana camara*, *Tridaxprocumbens*, and *Abutilon indicum* were collected from fields in and around Bangalore city.

### 1.2 Preparation of Plant Material

The leaves of the selected plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags.

#### 2.1 Methanol extraction of plant material

Plant leaf powder (10 g) was placed in a 250 ml conical flask containing 100 ml of methanol and plugged with cotton and placed on a rotary shaker (190-220 rpm for 24 h). Later, plant material-methanol mixture was filtered through 8 layers of muslin cloth and centrifuged at 5000 rpm for 15 min. The supernatant was collected and the solvent was evaporated to one-fourth of its original volume on water bath.

#### 2.2. Ethanol extraction of plant material

Ten gram (10g) of powdered plant material was taken in 250 mL conical flask containing 100 mL of ethanol (ethanol: water 80:20). The conical flask was covered tightly with an aluminum foil and placed on a rotary shaker (150 rpm for 24 h) for continuous agitation. The ethanol-plant powder mixture was filtered using muslin cloth followed by What man no 1 filter paper and vacuum to obtain the extract. The solvent from the extract was removed by warming on water bath temperature of 75°C. The residue collected was used for the experiments.

#### 2.3 Aqueous extracts of plant material

Powdered leaf (10 g) was homogenized with 50 ml of water and the suspension was heated to 50-60°C, and maintained for 15 minutes and filtered. The filtrate obtained was then centrifuged at 2500 rpm for 15 minutes to collect clear supernatant and stored at 5° C until further use.

### 3.0 Qualitative phytochemical analysis

Qualitative phytochemical analysis of plant leaf powder from *Adathodavisca*, *Daturastramonium*, *Lantana camara*, *Tridaxprocumbens*, and *Abutilon indicum*, was conducted following the standard procedures (Brinda et al., 1981).

**Test for Alkaloides :** Plant powder (20 mg) was taken and suspended into 10 ml of methanol. After 10 minutes the filtrate was collected. To determine alkaloids a 2 ml of filtrate was treated with 1 % HCL, and 6 drops of Mayer s reagent/Wagners reagent/ Dragendorffs reagent. The development of Creamish/Brown/Orange colored precipitate respectively indicated the presence of alkaloids.

**Test for Saponins:** 20mg leaf powder of each plant is boiled in 20ml of distilled water on a water bath and filtered.10ml of the filtered sample is mixed with 5ml of distilled water in a test tube and shaken vigorously to

obtain a stable persistent froth. The frothing is then mixed with 3 drops of olive oil and for the formation of emulsion which indicates the presence of saponins.

**Test for Tannins:** 0.5g of powdered sample of each plant was boiled in 20ml of distilled water in a test tube and filtered. 0.1% FeCl<sub>3</sub> is added to the filtered samples and observed for brownish green or a blue black colouration which shows the presence of tannins.

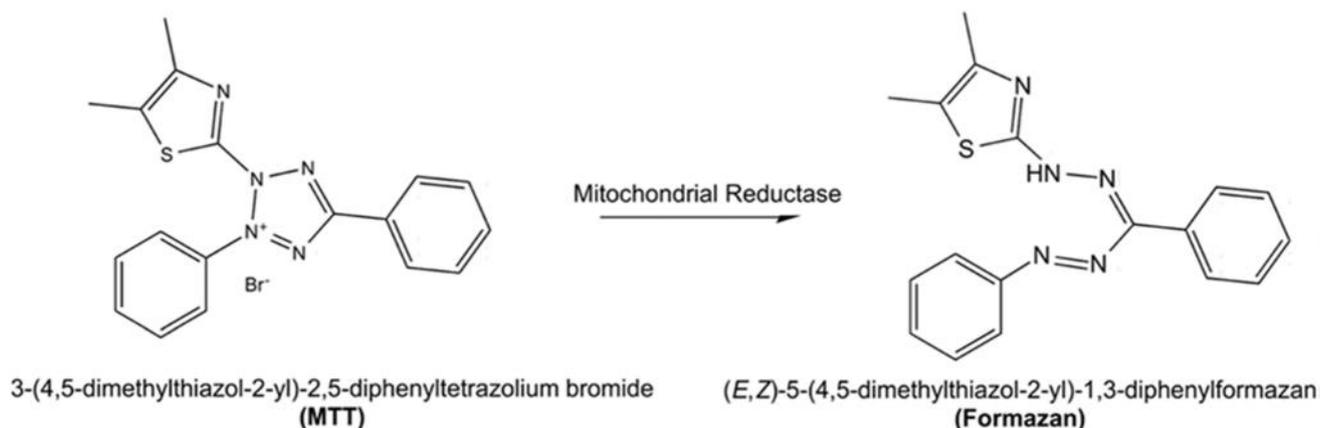
**Test for Flavonoid :** A few drops of 1% NH<sub>3</sub> solution is added to the aqueous extract of each plant sample in a test tube. A yellow coloration is observed if flavonoids are present.

### 3.1 Anticancer Activity

The anticancer activity of plant extracts was performed by MTT assay. The ability of the plant extract to destroy cancer cells has been tested on the basis that the conversion of yellow tetrazolium salt-MTT to purple-formazan crystals occurs only in metabolically active cancer cells. The population of viable cells is directly related to purple color of MTT thus proving quantitative determination of viable cells. The colour of the formazan is measured at 590 nm.

#### Procedure for MTT assay (cell viability assay) (Mossman, 1983)

The MTT assay is a colorimetric assay for measuring metabolically active cells. It is an enzyme, NAD (P) H-dependent cellular oxidoreductase based method. The MTT assay under defined conditions, represent the number of viable cells. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color.



#### Calculation of half maximal Inhibition Concentration - 50 (IC<sub>50</sub>)

The statistical, nonlinear regression method was adopted. The data are fitted by a method of successive approximation using Graph pad Prism6.

The percentage inhibition is calculated as follows:

$$\% \text{ inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

## RESULTS AND DISCUSSION

The anticancer activity of methanolic extracts was conducted against HCT116 cells line by MTT assay. The alkaloid fractions from all plant extracts have shown anticancer activity except *Adathodavisca* (Table 1).

**TABLE 1: EFFECT OF ALKALOIDS ON HCT116 CELLS.**

Samples	Conc. $\mu\text{g/ml}$	OD 590 nm	%Inhibition	IC <sub>50</sub> $\mu\text{g/ml}$
<i>Abutilon indicum</i>	Control	0.6898	0.00	104
	2.5	0.6721	2.57	
	5	0.6416	6.99	
	10	0.5921	14.16	
	20	0.5623	18.48	
	40	0.5399	21.73	
	80	0.4716	31.63	
	160	0.4109	40.43	
<i>Adathodavasicca</i>	2.5	0.6706	2.78	Not active
	5	0.6375	7.58	
	10	0.6147	10.89	
	20	0.5912	14.29	
	40	0.5412	21.54	
	80	0.5112	25.89	
	160	0.4764	30.94	
	320	0.4559	33.91	
<i>Daturastronium</i>	2.5	0.6345	8.02	57.23
	5	0.5879	14.77	
	10	0.5323	22.83	
	20	0.4483	35.01	
	40	0.3877	43.80	
	80	0.251	63.61	
	160	0.151	78.11	
	320	0.0647	90.62	
<i>Lantana camara</i>	2.5	0.6462	6.32	52.07
	5	0.5909	14.34	
	10	0.5465	20.77	
	20	0.4346	37.00	
	40	0.3834	44.42	
	80	0.2985	56.73	
	160	0.1725	74.99	
	320	0.0899	86.97	
<i>Tridoxprocumbons</i>	2.5	0.6648	3.62	131.3
	5	0.6468	6.23	
	10	0.6268	9.13	
	20	0.5941	13.87	
	40	0.5648	18.12	
	80	0.4907	28.86	
	160	0.4159	39.71	
	320	0.3389	50.87	

The effect of alkaloid fraction of leaf extract of five different plants on HCT 116 cell line are tabulated (Table 1). *Lantana camara* (IC<sub>50</sub> values 52.07 µg/ml) and *Datura stromonium* (57.23 µg/ml) extracts show potent anticancerous activity. *Tridax procumbens* and *Abutilon indicum* extracts showed moderate, but *Adathodaviscadid* did not show any anticancerous activity.

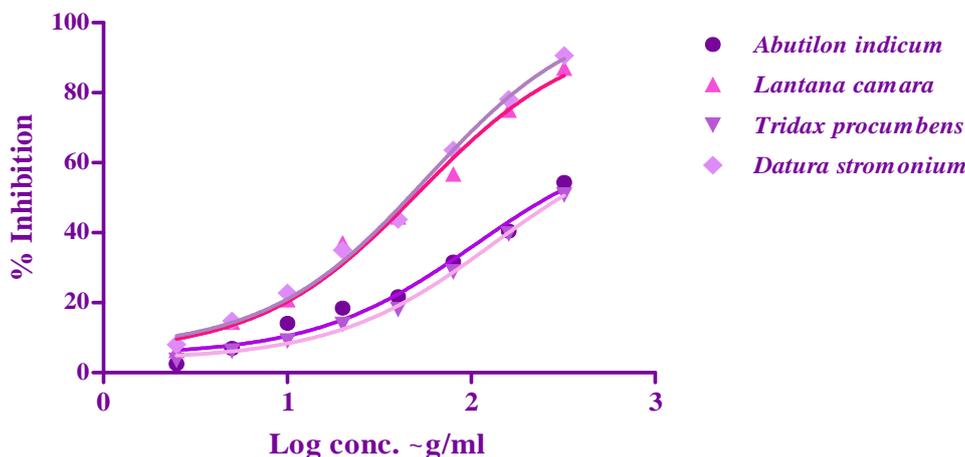


Fig. 1 Anticancer activity of alkaloids fraction leaf extract of four different plants on HCT 116 cell line (The values have corrected for control).

## 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 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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