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Evaluation of Antioxidant and Anticancer Properties of Methanolic Extracts of *Abutilon indicum* and *Blumea mollis*

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ABSTRACT

Successful plant remedies and their preparations as medicinal treatments have been used for thousands of years in indigenous cultures around the world. Many of today's valuable drugs such as asprin, digitoxin, morphine and quinine are resulted from traditionally used plants after scientific evaluation. In the present study medicinal plants namely *Abutilon indicum* and *Blumea mollis* were chosen to screen for potential anti-oxidant properties and cytotoxic activity. The concentration of phenol was determined using Folin-Ciocalteu assay (26) with minor modifications. The extract was also screened to assess the antioxidant activity using FRAP, 1, 1-Diphenyl-2-picrylhydryzyl [DPPH] radical scavenging activity and Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction (6) with slight modification. These extracts show anti-oxidant properties as well as inhibitory effect on cancer cells with the increased concentration and duration. When used in higher concentrations, these extracts show good inhibition rates which might prove to be effective for the prevention of cancer.

Keywords: *Abutilon indicum*; *Blumea mollis;* Antioxidant properties; Cytotoxic activity; DPPH; MEM

INTRODUCTION

Reactive oxygen species (ROS), such as superoxide anions, hydrogen peroxide, and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases (19, 20). Williams (22) reported that the body handles this cell damage process by producing a number of antioxidant enzymes which can be found both in the human body and in plants (23). Thus, antioxidants protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite.

Cancer kills annually about 3500 per million populations around the world. A large number of chemo preventive agents are used to cure various cancers, but they produce side effects that prevent their excess usage. Cancer has become a main interest of natural products chemistry over the years due to its widespread diagnosis. Natural products research has introduced many revolutionary compounds that have been used as ingestible remedies for a variety of health concerns and problems throughout the years. The importance of medicinal plants in the management of human ailments cannot be over emphasized. Natural products chemistry has brought forth compounds that have gone on to eventually lead to advanced cancer treatments, pain relievers, dietary. Since present medication and treatments producing side effects to the patients, there is a search for a traditional type of medication and can be proved as taxonomical markers (3, 14).

The medicinal plants harbor an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Natural products from medicinal plants are known to be chemically balanced, effective and least injurious with none or much reduced side effects as compared to synthetic medicines. Several bioassays are available to screen crude plant extracts for their potential for a specific biological activity. Therefore the main aim of this study is to determine the antioxidant and anticancer properties of methanolic extracts of *Abutilon indicum* and *Blumea mollis*.

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MATERIALS AND METHODS

Preparation, separation and concentration of plant extract:

Medicinal plants namely *Abutilon indicum* and *Blumea mollis* were authenticated. The air-dried and powdered whole plant materials were extracted thrice with methanol. The methanolic extract was concentrated under reduced pressure using the rotary evaporator at a temperature of 40°C. Concentrated methanolic extract were lyophilized to dryness using freeze drier. The powdered samples were stored at 4°C and used within seven weeks after production.

Antioxidant Studies:

Total Phenolic content determination assay:

Total natural phenolic of plant extracts was determined using Folin-Ciocalteu assay (26) with minor modifications. Test tube containing either 500ul of either standard solutions of gallic acid (50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125ug/ml) or crude extracts (diluted 400-fold with distilled deionized water) was prepared, 500ul of 10% Folin-Ciocalteu's phenol reagent (in DDW) was added into each test tube and mixed. After 20min, 350ul of 1M Na_2CO_3 solution was added into the mixture. After incubation for 20min at room temperature, the absorbance was determined at 750nm against the parallely prepared blank (500ul of DDW +500ul of 10% FC reagent + 350ul of 1M Na_2CO_3 solution).

Determination of DPPH radical scavenging activity:

The free radical scavenging activity of the extracts was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (2, 2-diphenyl-1- picrylhydrazyl). 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution

in water at different concentrations (250-2500 µg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH Scavenged (%) = (A control – A test / A control) × 100

Where **A** control is the absorbance of the control reaction and A test/std is the absorbance in the presence of the extracts. The antioxidant activity of the extracts was expressed as IC50. The IC50 value was defined as the concentration in µg/ml of extracts that inhibits the formation of DPPH radicals by 50%.

Reducing power assay:

The reducing power of methanolic extracts was determined according to the method of Oyaizu (1986). Different concentrations of pod extract (10– 100 µg/ml) in 1ml of methanol were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferrocyanide (2.5 ml, 1%). The mixture was incubated at 50oC for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl3 (0.5 ml, 0.1%) and the absorbance was measured at 700 nm and compared with standard. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of nitric oxide radical scavenging activity:

Nitric Oxide radical inhibition estimated by the use of Griess Illosvoy reaction (6). Nitric oxide was generated from sodium nitroprusside and measured by the Griess reaction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitric ions that can be estimated by use of Griess reagent. Scavenger of nitric oxide competes with oxygen leading leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate–buffered saline (PBS) was mixed with 3.0 ml of different concentrations (10-100 µg /ml) of the drugs dissolved in the suitable solvent systems and incubated at 25^0C for 150 min. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 2%H3PO4 and 0.1% napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm.

Anticancer studies:

Materials:

Serum containers, 15ml centrifuge tubes (TARSONS), 50ml Falcon (TARSONS), Glycerol, Minimal essential medium (MEM), Trypsin-EDTA, T25-flasks (Corning)**,** Tissue Culture Plates for MTT Assay (Nunc), MTT (SIGMA).

Culture Medium:

The culture medium (CM) consisted of MEM (Gibco) supplemented with 10% heat-inactivated fetal bovine serum (Gibco), 100 U/mL of penicillin and 100 mg/mL of streptomycin.

Cell lines and culture conditions:

Human cancer cell lines SK-MEL-28 (Melanoma) and NCI-H23 (Lung Adeno Carcinoma) were procured from National Centre for Cell Science, Pune. All cells were grown in Minimal essential medium (MEM, GIBCO). Cultures were maintained at 5% CO2 and 37°C temperature.

Cytotoxicity assay: NCI-H23 (3-5 × 10^3 per well) and SK-MEL28 (8-12 × 10^3 per well) cell lines were plated in 96-well plates in complete medium and treated in triplicate with different concentrations of plant extracts or 0.1% DMSO (control) for 72 hours. Cytotoxic potential of crude plant extracts on these cancer cell lines was estimated using MTT assay. The assay is based on the ability of dehydrogenases of living cells to convert 3-(4,5-dimethylthiazole-2-yl)-2,5-tetrazolium bromide (ÌÒÒ) of yellow color into insoluble purple-and-blue crystals of ÌÒÒ-formazan, which were extracted by isopropanol and determined on reader (Microplate Reader 2010, Anthos Biochrom) at ë=570 nm. Intensity of conversion ÌÒÒ>ÌÒÒ-formazan reflects the total level of dehydrogenase activity of cells, i.e. a final effect of cytotoxicity of injected preparation (28). MTT assay is advantageous in being rapid and precise without any radio isotope. Reduction of MTT takes place when reductase enzymes are active, and therefore conversion is often used as a measure of viable or living cells.

% cell survival: 100 – {(At-Ab) / (Ac-Ab)} × 100

Where: At = Absorbance of test,Ab = Absorbance of blank,Ac = Absorbance of control

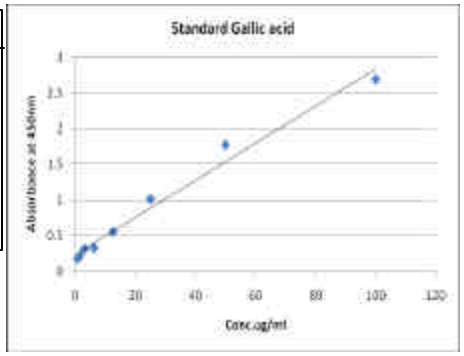
% **cell inhibition**: 100 - % cell survival

RESULTS AND DISCUSSION

In this study, DPPH, FRAP and Nitric oxide free radical scavenging were used to evaluate the antioxidant activity of methanolic extracts of *A. indicum* and *B. mollis.* The results show that the rate of free radical scavenging effect of *B. mollis* on DPPH was good especially that of *A. indicum* which was better than Ascorbic acid and BHT. A similar observation was made using different concentrations of the extract suggesting that the *A. indicum* and *B. mollis* possess strong antioxidant properties. The strong antioxidant activity demonstrated by the two species of *A. indicum* and *B. mollis* confirmed that they are free radical scavengers possibly as primary antioxidant that reacts with free radicals, particularly of the peroxy radicals, which are the major propagation of the autoxidation chain of fats, thereby terminating free radical chain reaction (23, 24, 25). This can be attributed to the antioxidant activity of the extract provided by the chemical constituents of the plant (26). Thus the mechanism may be derived from the action of the chemical compounds, flavonoids and phenols in the plant medicine (29). These findings therefore justify the use of these medicinal plants in the treatment of several ailments in India.

Total Phenolic content: Standard Gallic acid

Conc. In ug/ml	Abs. at 750nm
100	2.695
50	1.776
25	1.012
12.5	0.558
6.25	0.331
3.125	0.316
1.562	0.221
0.781	0.178



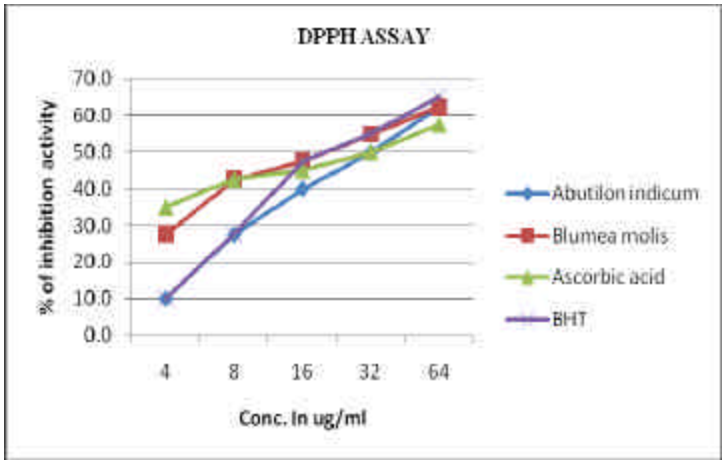
Total Phenolic content:

Abutilon indicum: 14.93ug GAE/mg

Blumea molis: 101ug GAE/mg

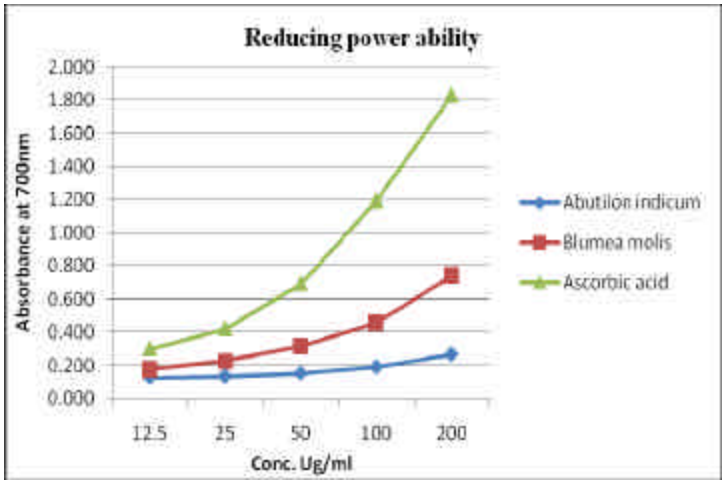
DPPH Radical scavenging activity

DPPH ASSAY % of Inhibition activity Conc. In ug/ml	Ab	Blu	Asc.	BHT
4	10.0	27.5	35	10
8	27.5	42.5	42.5	27.5
16	40	47.5	45	47.5
32	50.0	55	50.0	55
64	62.5	62.5	57.5	65



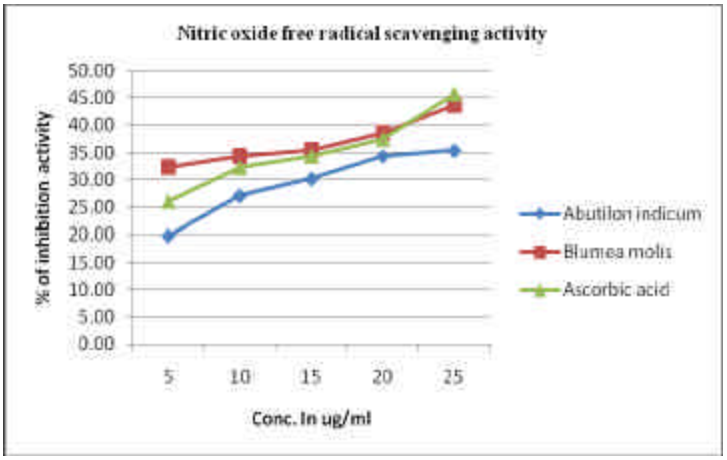
Reducing power ability:

Conc. In ug/ml	Absorbance at 700nm Ab	Blu	Asc.
12.5	0.129	0.176	0.300
25	0.134	0.224	0.422
50	0.153	0.318	0.695
100	0.191	0.456	1.194
200	0.268	0.744	1.830



Nitric oxide free radical scavenging activity

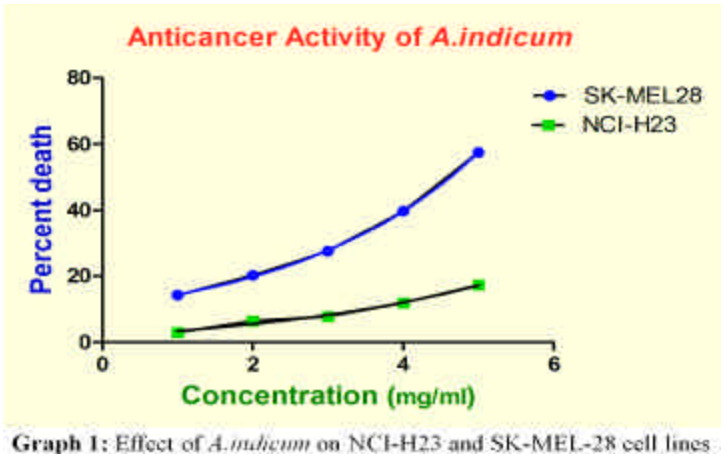
Nitric oxide free radical scavenging Conc. In ug/ml	Ab	Blu	Asc.
5	19.79	32.29	26.04
10	27.08	34.37	32.29
15	30.20	35.41	34.37
20	34.37	38.54	37.50
25	35.41	43.75	45.8



Methanolic extract of *A. indicum* was studied for inhibitory property on human melanoma (SK-MEL28 cell lines) and human non-small cell lung cancer (NCI-H23 cell lines) by MTT assay. *A.indicum* has shown IC_{50} value of 4.71 mg/ml on SK-MEL28 and IC_{50} value 15.8 mg/ml on NCI-H23 cell lines. Methanolic extract of *B. mollis* at 5mg concentration exhibited a high inhibition rate of 49.10% (IC_{50} value 6.31 mg/ml) on SK-MEL-28 cell lines and 57.84% inhibition rate at 5mg concentration (IC_{50} value 4.45 mg/ml) on NCI-H-23 cell lines.

Table 1: Shows the effect of *A.indicum* on SK-MEL-28 and NCI-H23 cell lines

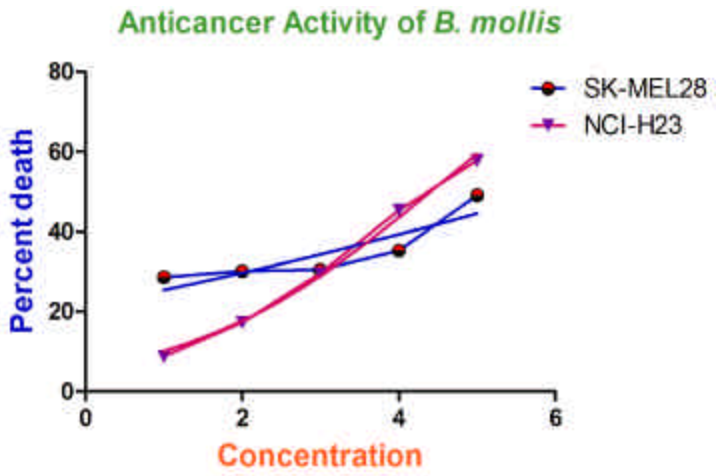
Concentration of *A.indicum*(mg/ml)	% of inhibition SK-MEL-28 cell line	NCI-H23 cell line	IC_{50} values SK-MEL-28 cell line	NCI-H23 cell line
1	14.26	2.94	4.71	15.8
2	20.21	6.52		
3	27.50	7.78		
4	39.79	9.90		
5	57.52	17.29		



Graph 1: Effect of *A.indicum* on NCI-H23 and SK-MEL-28 cell lines

Table 2: Shows the effect of *B.mollis* on SK-MEL-28 and NCI-H23 cell lines

Concentration of *B. mollis* (mg/ml)	% of inhibition SK-MEL-28 cell line	NCI-H23 cell line	IC_{50} values SK-MEL-28 cell line	NCI-H23 cell line
1	28.57	8.75	6.31	4.45
2	30.10	17.21		
3	30.44	29.55		
4	35.23	45.27		
5	49.10	57.84		



Graph 2: Effect of *B.mollis* on SK-MEL-28 and NCI-H23 cell lines

CONCLUSION

Previous studies on *Abutilon indicum* showed this plant contains Alanto lactone, isolactone and ß-sitosterol compounds which have anti-bacterial and anti-fungal properties while *Blumea mollis* with Linaloo and allo-aromadendrene has shown to possess anti-bacterial and anti-fungal properties. Both the medicinal plants exhibited varying sensitivity on cell lines on NCI-H23 and SK-MEL-28 cell lines. So, this study shows that these plants contain anti-cancer properties and can exhibit better response when used in higher concentration.

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