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IN VITRO ANTIOXIDANT AND CYTOTOXICITY ACTIVITIY OF *Bacopa monnieri* AND *Baliospermum montanum muell Arg.*

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ABSTRACT:

Alcoholic extracts of *Bacopamonnieri* and *Baliospermum montanum Muell Arg*. were screened for their possible antioxidant activity by DPPH free radical scavenging and cytotoxicity on proliferation of HT-29 colon cancer) cell line was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT micro culture tetrazolium viability assay. The cells were exposed to different concentrations (100, 50, 25, 12.5, 6.25and 3.125 µg/ml). In DPPH radical scavenging assay the % activity at100 (µg/ml) was 78.12 and 62.5 respectively and % cytotoxicity in MTT assay at100 (µg/ml) was 75.93 and 65.97 with IC50 of 35.5 and 47.14 respectively and R^2 value of the extracts are0.9987 0.9994 respectively. From the above results it was observed that alcoholic extract of *Bacopamonnieri* was more significant than the alcoholic extract of *Baliospermum montanum Muell Arg*.

KeyWords: DPPH, MTT assay, Cytotoxicity, IC_{50}, *Bacopamonnieri*, *Baliospermum montanum Muell Arg*

INTRODUCTION:

The incidence of colon cancer is rising in every country of the World. It is the fourth most common cause of cancer death (after lung cancer, stomach cancer and liver cancer). Thus, colon cancer is a worldwide disease and needs to be addressed seriously. Medicines derived from plants have played a pivotal role in health care of ancient and modern cultures. Ayurveda, the Indian system of medicine mainly uses plant based drugs or formulations to treat various ailments including cancer. Almost 60% of drugs approved for cancer treatment are of natural origin [1].There is always the hope that the search among the traditional medicinal plants may provide potent and safe medicines. *Bacopamonnieri Linn*. (Family-*Scrophulariaceae*) is a small, common, amphibious plant growing in marshy areas throughout the Indian subcontinent. Bacopais also called Brahmi, a name derived from Brahma, the creator god of the Hindu pantheon of deities. The triterpenoid saponins and their bacosides are responsible for Bacopa's ability to enhance nerve impulse transmission. Of synaptic activity and ultimately nerve impulse transmission [2].Traditionally, it was used as a brain tonic to enhance memory development, learning, concentration and to provide relief to patients with anxiety or epileptic disorders [3]. Research on anxiety, epilepsy, bronchitis and

asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of Bacopa [4]. Bacopa's antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer. It helps to prevent induced lipid peroxidation [5]. *In-vitro* research suggests an anticancer effect for *Bacopa* extracts, possibly due to inhibition of DNA replication in cancer cell lines [6]. (Wild) Muell. (Family-*Euphorbiaceae)* is an important medicinal plant, which is commonly called as Danti. The plant is a stout monoecious under shrub with many shoots from the base. The various parts of the plant like roots, leaves, and seeds are used traditionally for the treatment of various ailments. In Ayurveda, root are used to cure jaundice, leucoderma, skin diseases, wounds, and as an anthelmintic .The solvents and aqueous extract of only roots have been studied and found to possess anticancer, antimicrobial, free radical scavenging, immunomodulatory, hepatoprotective and anthelmintic properties [7]. Leaves are found to be useful in asthma, bronchitis [8] and in treating abdominal tumor [9]. The present study aimed to evaluate the possible Cytotoxic activity of the *Bacopamonnieri (*whole plant) and *Baliospermum montanum Muell Arg* (bark) alcoholic extracts used in the treatment of several diseases, but with no reports on its inhibitory effect on colon cancer potential of alcoholic extract of and respectively. Therefore, the aim of the present study was to evaluate the anticancer activity on HT-29 Human colon cancer cell line.

MATERIAL AND METHODS:

- Plant material collection: Collected from the ayurvedic shops in Belgaum. Plant and bark is identified and authenticated in Ghatprapha Ayurvedic College, Ghataprapha.
- The whole plant *Bacopamonnieri Linn* was dried in shade, under normal environmental conditions and then subjected to size reduction to get coarse powder and charged into the soxhlet apparatus and extraction was carried out with water and 95% ethanol.
- The bark of *Baliospermum montanum* was subjected to size reduction to get coarse powder and charged into the Soxhlet apparatus and extraction was carried out with water and 95% ethanol.

Cell Culture: Human colon cancer cell line (HT-29) obtained from the NCL PUNE India.

MTT assay:

MTT solution preparation: 10 mg in 10 ml of Hank's balanced solution.

Cell culture :

The cell line were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamycin, Penicillin (100 Units/ ml) and Streptomycin (100µg/ml) in presence of 5% Co2 at 37ºC for 3-4 days. After 3-4 days remove the supernatant and replace MEM media with Hank's balanced solution supplemented with Gentamycin, Penicillin and Streptomycin. Incubate overnight.

In-vitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. Remove the supernatant from the plate and add fresh Hank's balanced salt solution and treated with different concentrations of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. After 24 hrs incubation at 37ºC in a humidified atmosphere of 5% Co2, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4hr incubation. The supernatant carefully aspirated, the precipitated crystals of "Formazan blue'

were solubalised by adding DMSO (200µl) and optical density
was measured at wavelength of 570nm.
The test denotes the survival cells after toxic exposure. Percentage inhibition of the extract against all cell line was calculated using the following formula:

Surviving cells (%) =

$$\frac{\text{Mean OD of test compound}}{\text{Mean OD of control}} \times 100$$

% cell inhibition = 100 - % cell survival

The effects of extracts were expressed by IC_{50} values calculated from dose response curves. [10]

DPPH radical scavenging activity:

For assessment of DPPH radical scavenging activity DPPH solution was prepared by dissolving 4 mg DPPH in 100 ml methanol. A dilution series were prepared for ascorbic acid and extract. After that 5ml of sample solution was mixed with 0.5 ml DPPH solution and incubated for 30 min at room temperature in dark condition and absorbance was taken at 517 nm and calculated the % inhibition of DPPH radical [11].

$$\% \text{ inhibition of DPPH radical} = \frac{\text{Absorbance Control (Sample with DPPH – sample without DPPH)}}{\text{Absorbance of control}} \times 100$$

Calculations and statistics:

Results were expressed as percentage growth inhibition of control. IC_{50} values were derived from a nonlinear regression model (curvefit) based on sigmoidal dose response curve (variable) and computed using Graphpad Prism version 5.00.

Table 01: Cytotoxic Activity of *Bacopamonnieri* and Baliospermum montanum Muell alcoholic extract against HT-29 cell line.

S. No	Concentration (µg/ml)	Cytotoxic activity (%)		IC_{50} (µg/ml)		R^2	
		Bacopamonnieri Linn	*Baliospermum montanum Muell Arg*	*Bacopamonnieri Linn*	*Baliospermum montanum Muell Arg*	*Bacopamonnieri Linn*	*Baliospermum montanum Muell Arg*
1	100	75.93	65.97	35.5	47.14	0.9987	0.9994
2	50	62.76	48.95				
3	25	45.67	32.87				
4	12.5	33.35	20.03				
5	6.25	25.45	12.38				
6	3.125	25.37	10.44				

Fig.1: Cytotoxicity of Bacopamonnieri and Baliospermum montanum Muell extract against HT-29cell line

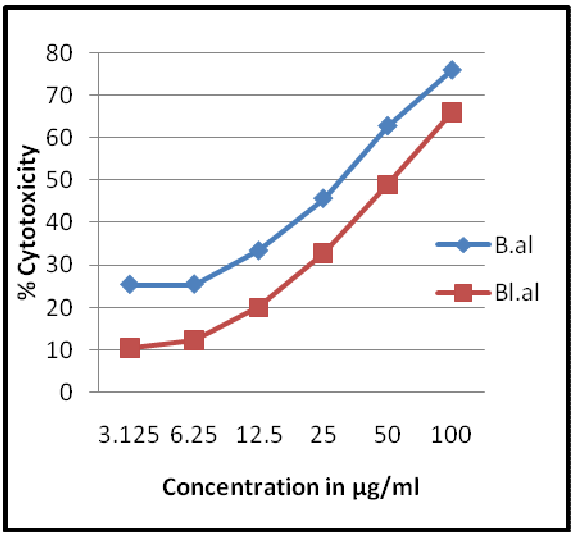
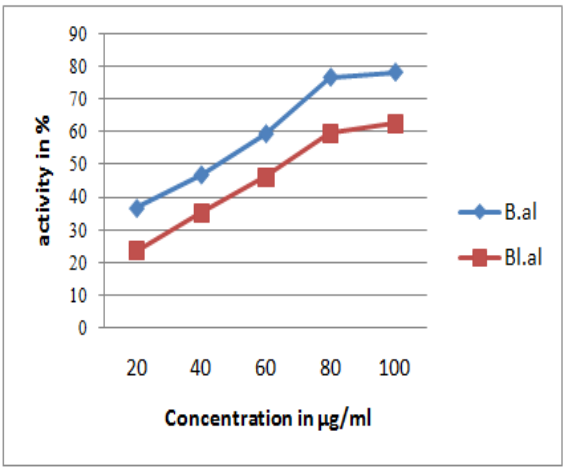


Table No.2: Effect of alcoholic extract of Bacopamonnieri and BaliospermummontanumMuell on DPPH

S.N0	**Concentration (µg/ml)**	**% Activity**	
		Bacopamonnieri Linn	*Baliospermum montanum Muell Arg*
1	20	36.7	23.43
2	40	46.87	35.15
3	60	59.37	46.09
4	80	76.56	59.37
5	100	78.12	62.5

Fig.2: DPPH(Radical scavenging activity)



RESULTS & DISCUSSION:

Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease [12]. Foods rich in antioxidants have been shown to play an essential role in the prevention of cardiovascular diseases, cancer, neurodegenerative diseases inflammation and problems caused by cell and cutaneous aging [13].

In the present study the cytotoxic activity of alcoholic extracts of *Bacopamonnieri* and Baliospermum montanum Muell Arg on HT-29 human colon cancer cell lines were evaluated with MTT assay. The cells were treated with various concentration of ethanol extracts, the relative cell survival progressively decreased in a dose dependent manner. The IC_{50} of alcoholic extracts was found to be 35.5 and 47.14µg/ml on HT 29, cell lines respectively. Among the tested extracts alcoholic extract was more selective cytotoxic against HT-29 cell line.

DPPH radical scavenging activity:

The results of this study clearly indicate that alcoholic extracts of *Bacopamonnieri* and Baliospermum montanum Muell Arg had significant scavenging effect on the DPPH free radical which increased with increasing concentration from 20-100 µg/ml. The percentage inhibition activity of aqueous and alcoholic extracts was 78.12 and 62.5 at 100 µg/ml concentrations respectively. The scavenging effect of sample was lower than that of Ascorbic acid. The extracts possess statistically significance DPPH free radical scavenging activity.

CONCLUSION:

In vitro cytotoxic activity against HT-29 cell line at different concentrations was evaluated. Cytotoxic effect *against* HT-29 colon cancer cell line is considered as a predictive anticancer activity indicator and IC_{50} value calculated for *both* extracts was below 50 µg/ml, which indicates that aqueous and alcoholic extracts of *Bacopamonnieri* and Baliospermum montanum Muell Arg potentially present an interesting cytotoxic activity and should be evaluated against primary cultures to determine the selectivity of their effects. We therefore, suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *invivo* and cytotoxic activity of these extracts to obtain useful chemotherapeutic agent. The antioxidant effect of these extracts may also contribute for the Cytotoxic activity.

REFERENCES:

[1] Grever, M.C.B, Cancer drug discovery and development. In: V.H.S. De Vita and S.A. Rosenberg Editors, Cancer: Principles and Practice of Oncology, 12.Sripathi, M., Sureban, S. Dharmalingam, R.Lippincott-Raven, Philadelphia, PA, 2001pp: 328-339.

[2] Singh HK, Dhawan BN. Neuropsychopharmacological effects of the Ayurvedic nootropic *Bacopamonniera* Linn. (Brahmi).*IndianJPharmacol*1997;29: S359-S365.

[3] Chopra RN. *Indigenous Drugs of India*. 2nd ed. Calcutta, India:U.N. Dhur and Sons; 1958:341.

[4] Shakoor A, Akram M, Asharaf CM and Siddiqui MR (1994)Pharmagonistic study and chemical /pharmacological evaluationofBrahmi-buti. HamdardMedicus. 37, 92-109.

[5] Tripathi Y B, Chaurasia S, Tripathi E, Upadhyay A and Dubey G P (1996) *Bacopamonnieri*Linn. as an antioxidant mechanism of action. Indian Journal of Experimental Biology 4(6): 523-526.

[6] Elangovan V, Govindasamy S, RamamoorthyN,Balasubramaanian K. *In vitro* studies on the anticancer activity of*Bacopamonnieri.Fitoterapia*1995; 66: 211-215.

[7] Ravindra G.M and Raju R.W *Baliospermum montanum* (Danti) Ethnobotany, Phytochemistry and Pharmacology- A review, Int. J. Green Pharmacy, Vol. 2 (4): (2008) 194-199.

[8] Nadkarni K. M. The Indian MateriaMedica, 3rd ed. Vol 1 Bombay: Popular Prakashan. (1988)

[9] Chopra R.N., Chopra I. C., Handi K.L and Kopor L.D. Indigenous drugs of India, Vol II, Academic Publishers, Calcutta. (1994)

[10] Cell and Tissue Culture For Medical Research Alan Dolly And J. Brayan Griffiths. Jhon Willey and Son's

[11] Mcdonald S, Prenzler PD, AutolovichM,Robards K. "Phenolic content and antioxidant activity of olive oil extracts." Food Chem ; 2001,73; pp73–84.

[12] Yodium K A & Joseph j a, A possible emerging role of phytochemicals in improving age-related neurological dysfunctions-a multiplicity of effects, Free Rad BIOL Med,30(2001)583.

[13] Pratt D E, Natural antioxidants from plant material , in phenolic compounds in foods and their effects on health II:Antioxidants and cancer prevention I(ACS Symposium series 507) edited by M Hang C.Ho& C Lee(American chemical society, Washington DC)1992, 54.