

ANTIPROLIFERATIVE AND ANTIOXIDANT ACTIVITY OF LEAVES EXTRACTS OF *MORINGA OLEIFERA*

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ABSTRACT

Objective: The aim of the study was to investigate the ethanolic and aqueous extract of leaves of *Moringa oleifera* for phytochemical constituents, antiproliferative and antioxidant activity.

Methods: The ethanolic extract of leaves of *Moringa oleifera*, belonging to the family Moringaceae was prepared by using soxhlet apparatus and aqueous extract was prepared by using maceration process. The extract was evaluated for its phytochemical constituents. The antiproliferative effects of both extracts were checked by using MTT ([3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide]) assay on HT-29 colon cell line and the antioxidant activity were checked by using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. In antiproliferative and antioxidant activity the 5-FU (5-fluoro uracil) and Ascorbic acid used as a standard drug for present results conclusion respectively.

Results: The results obtained in MTT assay shown that ethanolic extract of *Moringa oleifera* had a more potent antiproliferative effect (growth inhibition of 62.25% at 100 µg/ml) on HT-29 colon cell line as compared to aqueous extract (% growth inhibition of 27.86 at 100 µg/ml) of *Moringa oleifera*. The ethanolic extract of *Moringa oleifera* shown more potent antioxidant activity (% inhibition of ethanolic 75.57 at 100 µg/ml) than aqueous extract (38.16 at 100 µg/ml) of *Moringa oleifera*. The activity shown by the extract is concentration dependent.

Conclusion: In the present study we have investigated that the effect of ethanolic and aqueous leaves extracts of *Moringa oleifera* possess antiproliferative and antioxidant properties.

Keywords: Antiproliferative, Antioxidant, MTT assay, DPPH assay, HT-29 colon cell line

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INTRODUCTION

Cancer is the largest single cause of death in both men and women. The Cancer cells typically invade and destroy normal cells. The major causes of cancer are dietary imbalances, smoking, hormones imbalances and chronic infections leading to chronic inflammation [1]. In India, colorectal cancer frequency has been increasing in recent years because of change in lifestyle of diet and environment factors. The dietary is the one of the prevalence factors for colon carcinogenesis due to having high fat, high protein and low carbohydrate; low fibers are implicated as stir up agent in epidemiological and animal studies [2]. Colorectal cancer (CRC) is the third most common cancer in the world and the third leading cause of cancer-related mortality in the United States [1].

Mutation is to account for most cancer, including colorectal. The cascade of events proposed to account for the development of a neoplasm in normal colonic epithelial tissue and leads to multiple polyps [3]. Some evidence suggests that the diet is an important environmental factor in the development of colon cancer and high consumption of red meats, animal fats, chocolate, alcohol and refined cereals are linked to higher incidence of cancer [4]. Natural dietary antioxidants compounds such as fruits and vegetables protect against damages caused by reactive oxidants [5]. Hence in the present study, we selected vegetables as one of the anticancer agents on HT-29 cell line

Moringa oleifera known as Moringa is native to north India having the bioactive potency for antibacterial, antifungal and secondary metabolites which are frequently used in traditional medicine as a reference to its curative power in colon cancer [6] and some other diseases [7]. Leaves of *Moringa oleifera* serve as a valuable source of nutrient for all age groups and play a role in fighting malnutrition [8]. India has the prime position in the cultivation and production of *Moringa oleifera*. So researchers have become influenced that nutrients found in fruits and vegetables do more than just prevent

deficiency diseases [8] and a high consumption of fruits and vegetables reduced the risk of cancer [9].

The leaves are highly nutritious, which contain more vitamin A, calcium, iron, vitamin C and potassium. *Moringa oleifera* is rich in various phytochemicals like carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phyto-estrogens and phenolics in flowers, leaves, roots, fruits and seeds, etc [9]. Consideration into above data, in account of use as a folk medicine of *Moringa oleifera* and its prevalent claims of the medical effectiveness and the lack of experimental studies on its *in vitro* HT-29 cell line activity, hence the present investigation was undertaken to evaluate the antiproliferation and antioxidant activity of the aqueous and ethanolic extract of *Moringa oleifera* on HT-29 human colon cell lines.

MATERIALS AND METHODS

Plants

In the present study, the *Moringa oleifera* leaves were collected from the local area. The leaves were authenticated by Dr. S. Y. Jadhav, H. O. D of Botany. Yashwantrao Chavan Science College, Warananagar, Kolhapur, Maharashtra India, studies include organoleptic tests, and macroscopic and microscopic observations were done. Then voucher specimen is deposited at Science College and the number is YCS/BOT/29. Soon after authentication, the leaves were used for polar and nonpolar solvent extraction by using a soxhlet apparatus then extract was subjected for phytochemical investigation.

Cell culture

HT-29 human colon cancer cells were obtained from NCL, Pune, India. The cells were maintained in the laboratory. 2, 2 diphenyl picrylhydrazyl (DPPH), as a reagent was purchased from Sigma, dissolved to 5 mmol/l in DMSO as a stock solution. MTT reagent was purchased from Sigma. The two solutions were preserved at 4 °C.

Cell lines culture

In *in vitro* antiproliferative activity, the human colon cell lines (HT-29 colon) were tested. The cell lines were obtained from NCL, Pune, India. The cell lines were cultured in RPMI 1640 medium supplemented with 5% of fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin at 37 °C in a humid atmosphere containing 5% CO₂.

Antiproliferative assay

The cells (100 µl/well, at a density of 3-6 × 10⁴ cell/ml) were plated in 96-well plates and incubated with sample at various concentrations (0.25 to 250 µg/ml, 100 µl/well) in DMSO/RPMI 1640/FBS 5% at 37 °C and 5% of CO₂ in air for 48 h. Final DMSO concentration did not affect cell viability.

Cells were then fixed with 50% trichloroacetic acid, and cell proliferation was determined by spectrophotometric quantification of cellular protein content at 540 nm, using the MTT assay [10]. 5-fluorouracil (3.125-100 µg/ml) used as the positive control in this assay. Three measurements were obtained at the beginning of incubation (time zero, T₀) and 48 h post incubation for compound-free (C) and tested (T) cells.

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of Control} - \text{Mean OD of test}}{\text{Mean OD of control}} \times 100$$

$$\% \text{ cell inhibition} = 100 - \% \text{ cell survival}$$

The effects of extracts were expressed by IC₅₀ values calculated from dose response curves.

RESULTS

Table 1: Antiproliferation activity of aqueous and ethanolic extract of *Moringa oleifera*

S. No.	Concentration (µg/ml)	<i>Moringa Oleifera</i> (% Cytotoxic activity)		<i>Moringa Oleifera</i> IC ₅₀ (µg/ml)		<i>Moringa Oleifera</i> R ²		
		Aqueous	Ethanolic	5-FU	Aqueous	Ethanolic	Aqueous	Ethanolic
1	100	27.86	62.25	81.81	Very wide	59.63	0.982	0.870
2	50	17.59	54.31	73.63				
3	25	12.77	41.77	66.00				
4	12.5	9.5	29.18	59.00				
5	6.25	8.1	22.59	51.00				
6	3.125	4.9	18.81	43.69				

Table 2: Antioxidant activity of aqueous and ethanolic extract of *Moringa Oleifera*

S. No.	Concentration (µg/ml)	% inhibition of Standard ascorbic acid	% inhibition of aqueous extract of <i>Moringa Oleifera</i>	% inhibition of Ethanolic extract of <i>Moringa Oleifera</i>
1	20	64.17	22.90	44.27
2	40	68.65	27.48	50.38
3	60	71.14	29.00	54.96
4	80	78.60	33.58	68.70
5	100	87.56	38.16	75.57

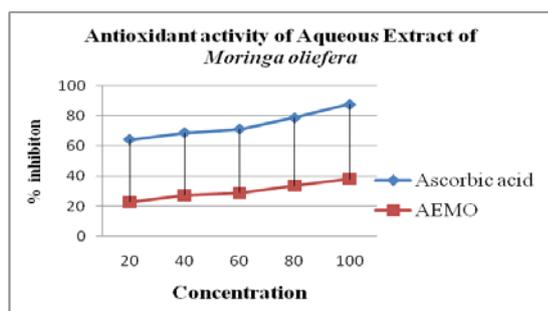


Fig. 2: It shows that the percentage inhibition in DPPH radical scavenging activity of *Moringa oleifera* aqueous extracts (20 to 100 µg/ml) compared to standard drug ascorbic acid. Values are expressed as inhibition Vs concentration

Antioxidant 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 5 ml 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].

$$\% \text{ inhibition of DPPH radicals} = \frac{\text{Absorbance of control} - \text{Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

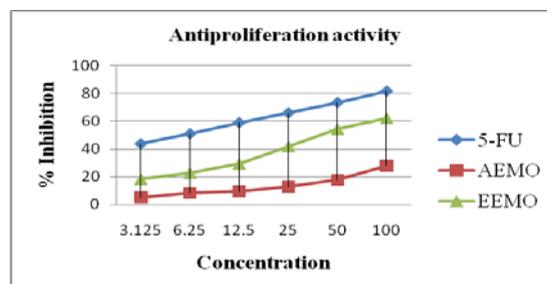


Fig. 1: It shows that the percentage inhibition or cytotoxicity in HT-29 human colon cancer cell lines of *Moringa oleifera* ethanolic and aqueous extracts (3.125 to 100 µg/ml) compared to 5-Fluoro uracil as a standard drug. Values are expressed as inhibition Vs Concentration

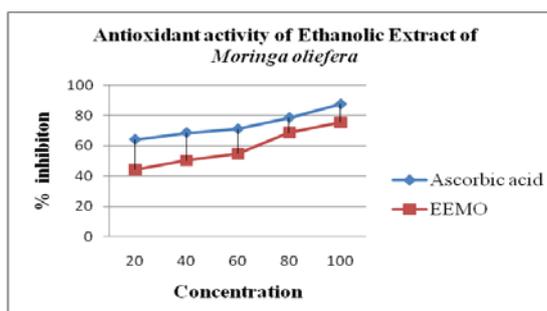


Fig. 3: It shows that the percentage in DPPH radical scavenging activity of *Moringa oleifera* ethanolic extracts (20 to 100 µg/ml) compared to standard drug ascorbic acid. Values are expressed as inhibition Vs Concentration

DISCUSSION

Indian system of herbal therapy is one of the most common therapies for different types of diseases and disorders. Nowadays the Researchers and Scientist has much more interested in demonstrating and discovering new molecule in plant extract due to plenty of potent antioxidant constituents present in the plant extracts. In the present study selected most common and easily available plant of *Moringa oleifera*, MTT assay and DPPH assay for antiproliferative and antioxidant activity on HT-29 colon cancer cell line respectively. The *Moringa oleifera* already has proven an anthelmintic [12], antibacterial [13], antitumor [14], and anticancer [15] pharmacological activity.

MTT assay was most widely used method hence in the present study the extracts were compared with standard drug 5-fluorouracil.

In Experiment the test samples mainly ethanolic extract of *Moringa oleifera* (EEMO) showing growth inhibition of 62.25% at 100 µg/ml was considered to be active. But an aqueous extract of *Moringa oleifera* (AEMO) showing % growth inhibition of 27.86 at 100 µg/ml was considered to be less active when compared with standard 5-fluorouracil. We concluded that the plant extracts showed *in vitro* cytotoxicity against HT-29 cancer cell line. The activity might depend upon the morphology of cell lines and mechanism of action of the plant extract and nature of the solvent used for extraction. Many plant extracts kills cancer cell lines through activating apoptosis and effecting growth regulators.

In biochemical systems, superoxide radical can be transformed into hydrogen peroxide by the action of superoxide dismutase and the H₂O₂ can subsequently generate particularly reactive hydroxyl radicals in the presence of certain metal ions. Hydroxyl radicals can attack DNA molecules to cause strand cleavage. The DPPH radical is scavenged by antioxidant through the donation of electron forming reduced DPPH. The above explanation is similar effective in ethanolic extract of *Moringa oleifera*, when compared to standard ascorbic acid in the DPPH assay. The present report depicted in the fig. (2 and 3) and table-2 were shown that the % inhibition of aqueous and ethanolic extract were 75.57 and 38.16 at 100 µg/ml respectively. So it was concluded that leaves of plant ethanolic extract *Moringa oleifera* have antioxidant activity and concentration dependent.

CONCLUSION

The leaves of *Moringa oleifera* contain active constituents of saponin, steroids, flavonoids, terpenoids and total phenolics shows an antiproliferative and antioxidant properties. Hence in our study, these active constituents play an important role as an anticancer. Comparing the standard drug 5-FU with ethanolic extract shows the potent effect on HT-29 cell line than aqueous due to the difference in extraction ability of solvents. Finally, the present study was concluded that ethanolic extract of *Moringa oleifera* has a potent antiproliferative and antioxidant activity.

CONFLICT OF INTERESTS

Declare none

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