Exploring *Oxystelma esculentum* R. Br.: A Potential Herbal Approach to Combat Oxidative Stress, a Leading Cause of Cancer

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Abstract

Oxidative stress produces detrimental effects on body leading to multiple diseases including cancer, neurodegenerative, and cardiovascular diseases. Cancer is a fatal and diverse group of disorders and its treatments like surgery, chemotherapy, and radiotherapy have side effects, like bone marrow depression, nephrotoxicity, affecting patients physically and emotionally, potentially causing social isolation. Nature has gifted human kind a variety of medicinal plants having antioxidant potential. *Oxystelma esculentum* is one of the important plants of traditional system of medicines. *O. esculentum* extract was tested using DPPH assay taking Ascorbic acid as control. Absorbance was recorded at 517 nm. Results of study proved that *O. esculentum* possess antioxidant potential in dose dependent manner. More over IC_{50} also showed good inhibition. Antioxidant activity of *O. esculentum* was studied by calculating free radical scavenging activity using DPPH assay. In this present study, *O. esculentum* was evaluated for cytotoxic, anti cancer activity by MTT colorimetric assay. *O. esculentum* showed excellent antioxidant potential as well as ant cancerous affect against MCF-7 cell lines thus can be applied to treat and prevent diseases caused by excessive oxidative stress.

Keywords

Oxystelma esculentum, Oxidative stress, Cancer, antioxidant

1. INTRODUCTION

Oxidative stress produces detrimental effects in the body and is a major cause of different ailments. Researchers have proven that increased oxidative burden not only initiate cancerous cell growth but it contribute in invigorating different diseases like diabetes, cardiovascular diseases, neurological problems like Alzheimer, Parkinson disease, delayed sexual maturation etc (Sharma and Singh, 2012).

Cancer is characterized by the uncontrolled growth of abnormal cells that can infiltrate and destroy normal tissues. While the exact cause is unknown, genetics, poor diet, infections, sedentary

lifestyle, obesity, tobacco use, and pollution are contributing factors. Free radicals play a significant role in cancer development. In 2023, cancer is estimated to have a higher mortality rate than coronary heart disease or stroke, with 14.1 million new cases and 8.2 million deaths worldwide in 2022, signaling a growing global cancer burden amid demographic and epidemiologic transitions (MacIntyre, M. F. 2015).

Current chemotherapy lacks ideal effectiveness due to MDR reversal agent toxicity. To address this, there's a crucial need for non-invasive and novel therapeutic strategies for treating debilitated cancer patients with severe mortality risks (Kornek, G. 2013).

Therefore, plants having antioxidant activity can promote cellular longevity thus play important role in decreasing the disease Burdon caused by oxidative degeneration (Pizzino *et al.* 2017; Kumar and Pandey, 2015).

Oxystelma esculentum belonging to the family Asclepiadaceae and the plant parts like leaves, bark, flowers and latex have been shown to possess various biological activities including, antibacterial, anti-ulcers, hepatoprotective, antitumor and antioxidant. Plant contains several bioactive compounds cardenolides, pregnane glycosides, flavonoids which are major classes of therapeutically important phytoconstituents. (Pandya, D. J., & Anand, I. S. 2011). In this study, we have focused on the evaluation of antioxidant and anticancer activities in vitro.

2. MATERIALS AND METHODS

The herb was collected from the water-lodged areas hashmahi canal Bahawalpur, leaves was separated and identified by Dr Ghulam Sarwar In charge, Department of Botany, Islamia university. The collected leaves were washed several several times with running water followed by deionized water to remove any dust particle. The shade dried leaves were ground into a fine powder. The extract was prepared by soaking 250 g powder in 500 mL of ethanol in a flask and dried via rotary evaporator and kept for 24h at room temperature with vigorous shaking.

2.1. Antioxidant Activity using DPPH Free Radical Scavenging Assay

Free radical scavenging activity of ethanolic extracts of *O. esculentum* were analyzed by DPPH assay following the procedure of (Shahidi, F., & Zhong, Y. (2015).

2.2. Sample preparation and Incubation

In this regard various concentrations of extract (100,250,and500mg/ml) were prepared and tested for antioxidant potential. Ascorbic acid (0.6mg/ml) was taken as standard and DPPH solution was used as blank.

All samples (test samples, standard, and blank) were freshly prepared and incubated for30 minutes prior to analysis.

% inhibition using following formula;

%inhibition = Absorbance of control - Absorbance of Sample / Absorbance of control * 100

2.3. IC_{50} value

IC_{50} values were calculated for each extract after assessment of % inhibition by plotting graph between Extract Concentration verse percent inhibitions.

2.4. Cytotoxic activity

MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-

diphenyl - tetrazolium bromide) colorimetric assay in 96 - well flat - bottom micro plates (Cloos, J. 2011) were used to assessed it's in vitro anti cancerous and cytotoxic activity. Following cell line used which include MCF-7 (breast cancer). For cell culturing Dulbecco's Modified Eagle Medium (DMEM) was used, which includes 5 % fetal bovine serum (FBS), penicillin (100 IU/ml) and streptomycin (100µg/ml) kept at 37ºC with 5% CO_2 incubation in75cm^2 flasks. Growing cells were gathered and counted via hemocytometer. In a certain medium all cells were diluted. 100 µl / well of cell culture in concentration of 1x10^5 cells / ml was place in 96 -well plates and incubate for overnight. Later, media was decanted and fresh media of 200 µl was poured with several concentrations of *O. esculentum.*

(50µg / ml was first prepared in DMSO and later diluted serially). After 72 hours, 50µl of MTT (2mg / ml) dye was added into each well and incubated it for further 4 hours. Later on, 100 µl of DMSO was decanted in each well of the plates and then incubated for 2 hours at 37°C.

Absorbance at a wave length of 570 nm, with the help of micro plate reader (Spectra Max plus, Molecular Devices, CA, USA).The assay method was directed in the triplicate. Doxorubicin was used as positive control. However, Phosphate Buffered Saline (PBS) used as a negative control.

The formula for percent inhibition

% Inhibition = 100 - [(A_e - A_b) / (A_c - A_b)] *100]

Where, A_e, A_b and A_c indicate mean Absorbance of extract, control and standard respectively. From % inhibition results parallel IC_{50} values were proved by plotting graph.

3. RESULTS AND DISCUSSION

Oxidative stress is found as a progressive cause of not only cancer but also different chronic diseases like diabetes, cardiac and neurodegenerative diseases especially age related complications.

Human body is equipped with different natural anti oxidant like glutathione, ascorbic acid (vitamin C), to copherol (vitamin E), superoxide dismutase etc. that provide defensive mechanism against peroxides or free radicals. Inhibition of free radicals ultimately prevents progression of diseases. Body's natural anti oxidant potential can be strengthen by utilizing medicinal plants having anti oxidant Phyto chemicals (Shareef *et al.,* 2014).

In this research study *Oxystelma esculentum* has been assayed for free radical scavenging activity by DPPH method. The plant material was extracted with ethanol. Antioxidant profile of extract of *O. esculentum* at different concentrations (100, 250, and 500 mg/ml was generated via calculating its respective % free radical scavenging activity (% inhibitions value). Results proved that this plant possess antioxidant potential in dose dependent manner, as results of anti oxidant potential of *O. esculentum* at doses of 100, 250, and 500 mg/ml keeping Ascorbic acid as Standard shown that maximum inhibitory effect was detected 89% at dose of 500mg/ml (Table1).

Table 1. Antioxidant potential of *Oxystelma esculentum*

Sample	% Inhibition	IC_{50} Values
Extract 100 mg	89	44
Extract 250 mg	78	
Extract 500 mg	57	
Ascorbic acid	91	

3.1. Cytotoxic effect of *Oxystelma esculentum*

IC_{50} values obtained against MCF-7 (breast cancer cell line) by *O. esculentum* and doxorubicin were 28.39 ±1.3ìg / ml thus corresponding to positive antiproliferative activity against MCF-7cell line in our study (Table2).

Table 2.Results of in vitro cytotoxic assessment against MCF-7 cell line

Sample	% Inhibition	IC_{50} Values
Extract 100 mg	51.1	28.39±1.3µg/ml
Extract 250 mg	63.5	
Extract 500 mg	71.65	
Doxorubicin	78.9	

Number of experiments (n) = 3,Values are IC_{50}, ± Std. Error of Mean

MTT assay is reproducible, sensitive and simple technique. MTT is a yellow color salt which reduces to form a crystals of violet color in metabolically active cells by help of enzyme succinate dehydrogenize which shows mitochondrial activity. MTT assay has multiple application like cell growth, cell viability, cell toxicity, cell activation and sensitivity of drug conversely generally used for the purpose of compounds having cytotoxic effect (Barham *et al.*, 1972). According to our results, Oxystelma esculentum showed growth inhibitory effect against cancerous cell line (MCF-7) by using MTT assay.

4. CONCLUSION

Oxystelma esculentum have been proved as free radial scavengers showing strong antioxidant activity. This medicinal action render a potential candidate to be used in forth prevention and treatment of diseases caused by increased oxidative stress like cancers etc. and is need to study the mechanistic action of observed anticancer potential.

5. REFERENCES

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