



INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND NOVEL SCIENCES

IJPRNS

IN-VITRO ANTIOXIDANT AND ANTICANCER POTENTIAL OF ETHANOLIC EXTRACT OF *SOLANUM SURATTENSE*

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ABSTRACT

To explore the preliminary phytochemical screening, *in-vitro* pharmacological evaluation of antioxidant activity and anticancer potential of ethanolic extracts of *Solanum surattense*. Ethanolic extract of the *Solanum surattense*, a widely used medicinal herb in traditional medicine, demonstrated potential, dose dependent, cytotoxic activity against HeLa cancer cell lines

Key Words: *Solanum surattense*, cytotoxic activity, antioxidant activity

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INTRODUCTION

Cervical cancer is a cancer arising from the cervix.[1] It is due to the abnormal growth of cells that have the ability to invade or spread to other parts of the body.[11] Early on, typically no symptoms are seen. Later symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during sexual intercourse.[1] While bleeding after sex may not be serious, it may also indicate the presence of cervical cancer. Human papillomavirus (HPV) infection causes more than 90% of cases;[4][5] most people who have had HPV infections, however, do not develop cervical cancer.[2] Other risk factors include smoking, a weak immune system, birth control pills, starting sex at a young age, and having many sexual partners, but these are less important.[1][3] Cervical cancer typically develops from precancerous changes over 10 to 20

years.[2] About 90% of cervical cancer cases are squamous cell carcinomas, 10% are adenocarcinoma, and a small number are other types.[3] Diagnosis is typically by cervical screening followed by a biopsy. Medical imaging is then done to determine whether or not the cancer has spread.[1] HPV vaccines protect against between two and seven high-risk strains of this family of viruses and may prevent up to 90% of cervical cancers.[14][7] As a risk of cancer still exists, guidelines recommend continuing regular Pap smears.[7] Other methods of prevention include: having few or no sexual partners and the use of condoms.[6] Cervical cancer screening using the Pap smear or acetic acid can identify precancerous changes which when treated can prevent the development of cancer. Treatment of cervical cancer may consist of some combination of surgery, chemotherapy, and radiotherapy.[1] Five year survival rates in the United States are 68%.[8] Outcomes, however, depend very much on how early the cancer is detected.[3]

MATERIALS AND METHODS

In vitro antioxidant activity of the extracts

The prepared extracts were tested for *in vitro* antioxidant activity using standard methods. The concentrations of the sample and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812, up to 0.025 µg/ml. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated.

Radical scavenging activity by DPPH method

2, 2 - diphenyl - 1 - picryl - hydrazyl (DPPH) is widely used to test the ability of compounds as free radical scavengers or hydrogen donors. Samples of different concentrations were prepared by dissolving in DMSO. 100 µl of either the samples or the standard solution was taken separately in test tubes and 2 ml of DPPH solution (0.1 mM) was added. The test tubes were incubated at 37°C for 30 min and the absorbance of each solution was measured at 490 nm [9]. A control in these experiments was prepared by same protocol except that the compound was not included.

Scavenging of ABTS [2, 20-azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) diammonium salt] radical cation assay

Accurately weighed 54.8 mg of ABTS (2, 20-azino-bis (3-ethylbenzo-thiazoline-6-sulphonic acid) was dissolved in 50 ml distilled water (2 mmol) and potassium persulphate (17 mmol, 0.3 ml) was added. The reaction mixture was left to stand at room temperature overnight in the dark before use. To 0.4 ml of various concentrations of the sample or standards, 2.0 ml distilled dimethyl sulphoxide (DMSO) and 0.32 ml of ABTS solution were added to make a final volume of 2.72 ml. Absorbance was measured after 20 min at 734 nm.

MTT assay method

Traditionally, the determination of cell growth is done by counting viable cells after staining with a vital dye. Several approaches have been used in the past. Trypan blue staining is a simple way to evaluate cell membrane integrity (and thus assume cell proliferation or death) but the method is not sensitive and cannot be adapted for high throughput screening.

Measuring the uptake of radioactive substances, usually tritium-labeled thymidine, is accurate but it is also time-consuming and involves handling of radioactive substances. Yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is reduced to purple formazan in the mitochondria of living cells. The absorbance of this colored solution can be quantified by measuring at a certain wavelength (usually between 500 and 600nm) by a spectrophotometer. The absorption max is dependent on the solvent employed. This reduction takes place only when mitochondrial reductase enzymes are active, and therefore conversion can be directly related to the number of viable (living) cells. When the amount of purple formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve. Solutions of MTT solubilized in tissue culture media or balanced salt solutions, without phenol red, are yellowish in color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring, yielding purple MTT formazan crystals which are insoluble in aqueous solutions. The crystals can be dissolved in acidified isopropanol. The resulting purple solution is spectrophotometrically measured. An increase in cell number results in an increase in the amount of MTT formazan formed and an increase in absorbance. The use of the MTT method does have limitations influenced by: (1) the physiological state of cells and (2) variance in mitochondrial dehydrogenase activity in different cell types. Nevertheless, the MTT method of cell determination is useful in the measurement of cell growth in response to mitogens, antigenic stimuli, growth factors and other cell growth promoting reagents, cytotoxicity studies, and in the derivation of cell growth curves. The MTT method of cell determination is most useful when cultures are prepared in multiwell plates. For best results, cell numbers should be determined during log growth stage. Each test should include a blank containing complete culture medium without cells [10].

The IC₅₀ value of ethanolic extract was observed at a concentration of 11.5µg/ml in DPPH method (Table-1).

Table-1 *In vitro* antioxidant activity of ethanolic extract of *Solanum surattense* by DPPH method

Sample/standards	IC ₅₀ value ± SE (µg/ml) by methods * DPPH method
Ethanolic extract	11.50±0.32
Ascorbic acid	0.95±0.35

*Average of three determinations (n=3)

In the present study, we applied the MTT test to evaluate the cytotoxic effect of ethanolic extracts of *Solanum surattense* (EES). Value is generated from the dose-response curves for *Helacell* line. The cancer cells were exposed to increasing concentrations (12.5-300 µg/mL) of ethanolic extracts of *Solanum surattense* for 48 h. The MTT assays with corresponding IC₅₀ are summarized in Table-2.

Table-2 *In vitro* cytotoxicity assay of ethanolic extract of *Solanum surattense* against the Hela Cell lines

Sample Concentration (µg/mL)	% Cell viability	% inhibition
Control	100±0.0	00
12.5	86.4±10.9	13.6
25	74.1±6.79	25.9
50	68.3±5.54	31.7
75	52.60±7.62	47.4
100	43.1±3.92	56.9
150	37.4±8.83	62.6
200	30.2±6.19	69.8
250	24.2±3.93	75.8
300	20.4±2.50	79.6

CONCLUSION

Phytochemical analysis of whole plant of ethanolic extract of *Solanum surattense* showed the presence of different phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, proteins,

Carbohydrates, coumarins & phytosterols. The ethanolic extract of the *Solanum surattense*, a widely used medicinal herb in traditional medicine, demonstrated potential, dose dependent, cytotoxic activity against HeLa cancer cell lines with IC₅₀ values of 103.6µg/mL.

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