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Research Article

IN VITRO ANALYSIS OF ANTICANCER POTENTIAL OF *TRIGONELLA FOENUM-GRAECUM*

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ABSTRACT

Objective: The recent work was carried out to evaluate the presence of bioactive compounds and anticancer activities of selected plant *Trigonella foenum-graecum* extract.

Methods: The crude extraction of whole plant was carried out using petroleum ether as a solvent by cold percolation as well as hot percolation method (Soxhlet method) both. The presence of phytochemicals was determined using standard protocols. The anticancer activities were evaluated by sulforhodamine B (SRB) dye assay method using Mitomycin-C (anticancer drug) as a positive control.

Results: The qualitative analysis of plant extract showed the presence of bioactive compounds, namely, protein, alkaloids, steroids, phenolics, and flavonoids. The plant extract was tested for *in vitro* anticancer activity against human liver cancer (HEP-2) and colon cancer (HT-29) cell lines using SRB assay. The plant extract showed significant results against HT-29 and HEP-2 cancer cell lines.

Conclusion: The study concluded that the extract of *T. foenum-graecum* can be further carefully used in herbal formulations for cancer therapy.

Keywords: Synthetic, Anticancer, *Trigonella foenum-graecum*, Herbal, Flavonoids.

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INTRODUCTION

Cancer is a heterogeneous group of diseases that affect people at all ages and can spread to surrounding tissues. The American Cancer Society reported that the deaths due to cancer constitute 2–3% of the annual deaths worldwide. Normally, the balance between cell division and cell death is tightly regulated, but disturbances in this process cause either a benign or a malignant tumor [1]. The study of research papers/articles clearly indicated that herbal medicine exhibits a variety of therapeutic properties and provides more health security to the people in primary health care [2-5]. According to the WHO, about more than 80% of the human population relies on Ayurvedic medicines for their primary health care [6]. The past review literature showed that various parts of the plant were used in Ayurveda, Unani, and Siddha for the treatment of various kinds of human and animal diseases [7]. In the area of herbal treatment, *Trigonella foenum-graecum* L. have rich source of proteins, phenolics, alkaloids, flavonoids, steroids, etc., that have a potential to the treatment in various types of diseases including cancer [8]. Herbal plants are being used traditionally for the treatment of various kinds of illness [9]. In recent time, there is a great emphasis which has been given toward herbal research on complementary and alternative medicine that deals with the treatment and cure of cancer [10]. The medicinal plants have a diverse group of highly valuable and readily available source of bioactive organic compounds, for example, alkaloids, tannins, essential oils, proteins, and flavonoids [11-13] which have been used in medicinal practices for a long time [14]. The anticancer activity of the plant *Tectaria cicutaria* extract was tested *in vitro* using sulforhodamine B (SRB) assay against some cancer cell lines, namely, human leukemia cell line (K-562), human nasopharyngeal cell line (KB), human colon cell line (HT-29), and human colon cell line (COLO-205) that showed significant results [15].

T. foenum-graecum L. is an annual herb crop commonly known as fenugreek which belongs to Family *Fabaceae.* The fenugreek seeds are widely used in South Asia for lowering the blood sugar level, fatty acids, and cholesterol levels. Hence, it is used in the prevention/treatment of diabetes and coronary heart diseases [16]. In ancient Rome, the roots of *T. foenum-graecum* were used to aid labor and delivery. In traditional Chinese system of medicine, *T. foenum-graecum* seeds were used as a tonic as well as the treatment for weakness and edema of legs [17]. *T. foenum-graecum* extract composed of proteins, polysaccharides, oils, fibers, flavonoids, saponins, trigonelline, and choline [18].

Keeping the above review literature in mind, our research work was, therefore, planned to evaluate the presence of bioactive compounds and anticancer potential of *T. foenum-graecum* extract using two selected human cancer cell lines, namely, liver cancer cell line (HEP-2) and colon cancer cell line (HT-29).

METHODS

Collection of plant material, authentication, and preparation of plant extract

The plants were collected and authenticated from Botanical Survey of India (BSI), Dehradun, U.K., India. According to BSI, the botanical name of selected plant is *T. foenum-graecum* L. which belongs to the family *Fabaceae* with accession no. 114535. The whole plants were washed with tap water and dried at room temperature in the laboratory and then little grinded. The extract was obtained by cold percolation as well as hot percolation (Soxhlet) method, using petroleum ether as a solvent. Extract was fine filtered, dried, and frozen at 8°C. During experiment, the extract was redissolved in dimethyl sulfoxide (DMSO) to form stock solutions, which were filtering sterilized (0.2 µm) before testing the experiments.

Protocol for preliminary qualitative screening of bioactive compounds

The biochemical tests for the determination of bioactive compounds have been done using standard protocols. For each of the test, 100 µg/ml concentration of the extract was used [19].

Test for protein

One milliliter extract solution was treated with few drops of concentrated nitric acid, the formation of yellow color indicates the presence of proteins.

Test for alkaloids

Extract was dissolved individually in dilute HCl and filter with filter paper, then filtrate was treated with saturated picric acids solution resulting in the formation of light brown precipitate which indicates the presence of alkaloids.

Test for steroids

One milliliter extract mixed with 2 ml of chloroform then carefully added H_2SO_4, the formation of reddish-brown color indicates the presence of steroids.

Test for flavonoids

NaOH test

One hundred microliters extract solution was treated with few drops of sodium hydroxide solution, then the formation of yellow color was the result which becomes colorless on addition of dilute HCl acid indicates the presence of flavonoids.

Lead acetate test

To 1.0 ml of plant extract solution very carefully add freshly prepared few drops of lead acetate solution. The formation of light yellow precipitate indicated the presence of flavonoids.

Test for phenols

One hundred microliters extract solution was mixed with 2 ml of 2% solution of FeCl3. Blue/green color result indicated the presence of phenols.

Test for tannins

One hundred microliters extract solution was mixed with 2 ml of 2% solution of FeCl3. Black color indicated the presence of tannins.

Test for terpenoids

One hundred microliters extract solution was mixed with 2 *ml* of chloroform, then very carefully add 2 ml of conc. H_2SO_4 acid and shaken by rotating the test tube gently. The color of solution changed into reddish-brown that indicated the presence of terpenoid compounds.

Study of anticancer potential assay from extract

Sources of cancer cell lines

Cancer cell lines were obtained from the National Centre for Cell Sciences, Ganeshkhind, Pune, 411007 (India), for the recent experiments.

Human cancer cell lines

The selected human cancer cell lines such as liver cancer (Hep-2) and colon cancer (HT-29) were grown in RPMI-1640 media with 2 mM L-glutamine medium (pH 7.2). Penicillin tablet (antibiotics) was dissolved in PBS and sterilized by filtering through 0.2 µ cellulose filter paper in laminar airflow hood. The growth media were stored at low temperature (nearly 4°C). The whole complete growth media contained 10% fetal calf serum (FCS). The growth medium used for cryopreservation contained 20% FCS and 10% DMSO in growth medium. The cancer cell lines (Hep-2 and HT-29) were maintained at 37°C in atmosphere containing 5% CO_2 and 90% humidity [20].

***In vitro* study of anticancer potential**

The anticancer activity was determined by evaluating the cytotoxic potential of the test material using human cancer cell lines such as HEP-2 and HT-29, these test materials were allowed to grow on tissue culture plates in the presence of desired test material. The cell growth was determined using ELISA reader after staining with SRB dye which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells.

Preparation of cell suspension for assay

Human cancer cell lines (HEP-2 and HT-29) were grown in multiple tissue culture flasks (TCFs) at 37°C in the atmosphere of 5% CO_2 and 90% relative humidity in the presence of complete growth medium to obtain enough number of cancerous cells. The TCFs with cells at subconfluent growth stage were selected for the experiment. The fully grown cancer cells were carefully harvested after treatment with trypsin-ethylenediaminetetraacetic acid solution and then separated to single cell by smooth pipetting. Thereafter, viable cells were counted using Trypan blue dye with the help of hemocytometer. The cancer cell viability at this stage was found more than 97%. Viable cancer cell density was maintained and adjusted to 5000–40,000 cells/100 µl depending on the type of cell line. Cell suspension (100 µl) together with 100 µl of complete growth medium was added into each 96-well plate. The 96-well plates were kept in CO_2 incubator at 37°C for 24 h in an atmosphere of 5% CO_2 and 90% relative humidity inside. After 24 h, the test material, DMSO (vehicle control), and positive control were added [21].

SRB assay

The antiproliferative SRB assay was performed to assess growth inhibition. It is a colorimetric method assay which estimates the total cell number indirectly by staining cellular active proteins by SRB dye [22]. The microtiter tissue culture plates were removed out after 48 h incubation of the cells with test materials and gently layered with ice-cold 50% TCA in entire wells; then, 96-well tissue culture plate was incubated at 4°C for up to 1 h. After 1 h incubation, all cells were attached to the bottom and supernatant was discarded by simple pipetting. The cell plates were washed many times with double-distilled water to remove extra TCA, growth medium, low-molecular-weight metabolites, serum proteins, and other unwanted materials. After removing the tissue culture plate, it dried in air and then SRB solution was added very carefully into each well and then incubated about ½ h at room temperature. The unbound SRB solution was removed by washing all the wells of tissue culture plate with 1% solution of acetic acid and air dried. When the wells were dried, then 100 µl Tris buffer of pH 10.4 was added. The whole contents were mixed using mechanical shaker. After removing from mechanical shaker, the optical density (absorbancy) was measured on ELISA reader at 515 nm [23].

Positive control

The positive control used was Mitomycin-C (anticancer medicine).

Calculation: Cell viability and growth in the presence of test material were calculated as follows: Percentage cell growth in the presence of test material:

=(Cell growth in presence of test material/Cell growth in absence of test material)×100.

Percentage cell growth inhibition in the presence of test material was determined as follows:

=100 − percentage cell growth in presence of test material.

Criteria for the determination of activity

The test sample showing growth inhibition of ≥70% when solution concentration is 100 µg/ml, then it considered to be active.

Statistical analysis

The experimental result was obtained as the mean ± S.D. Differences were considered statistically significant when $P \leq 0.05$. Statistical calculations were carried out using Microsoft Excel 2010.

RESULTS

Qualitative screening of bioactive compounds

The quantitative biochemical tests from petroleum ether extract of *T. foenum-graecum* showed the presence of proteins, phenolics, alkaloids, flavonoids, and steroids (Table 1).

Result of *in vitro* anticancer activity

The anticancer activities of plant extract are summarized in Table 2.

If the test sample shows growth inhibition ≥70% at 100 µg/ml solution, then it is considered a significant result. The total experimental data were summarized in tabular form (Table 2) and graphs were plotted, as shown in Fig. 1 and 2. *In vitro* cytotoxicity of plant extract was determined against human liver cancer cell line (HEP-2) which showed 82%, 80%, and 72% growth inhibition of the sample number – UTU 4, UTU 10, and UTU 16, respectively, while Mitomycin-C (positive control) showed 82% growth inhibition, whereas in case of colon cancer cell line (HT-29) showed 77%, 78%, 78%, and 78% growth of inhibition of the sample number – UTU 9, UTU 11, UTU 16, and UTU 17, respectively, while Mitomycin-C (positive control) showed 80% growth inhibition. The mean values of all data showed 78% growth inhibition against HEP-2 cell lines while 77.75% growth inhibition against HT-29 cell lines [15].

DISCUSSION

The qualitative test results showed that *T. foenum-graecum* extract contains bioactive compounds, namely, proteins, phenolics, alkaloids, flavonoids, and steroids [18]. The anticancer potential was determined by the cytotoxic potential of the test material using liver and colon cancer cell lines (HEP-2 and HT-29), which were grown on tissue culture plates in the presence of test material. The cell growth is measured on ELISA reader after staining with SRB dye that binds to basic amino acid residues in trichloroacetic acid fixed cells. Table 1 shows that the *T. foenum-graecum* L. have rich source of bioactive organic compounds such as proteins, phenolics, alkaloids, flavonoids, and steroids that possess unique medicinal activities. Therefore, plant containing these bioactive organic compounds may serve as a potential source in the treatment of colon and liver cancers. The possible cause of cancer activity is due to the free radical formation. Hence, plants containing phenolics, alkaloids, flavonoids, steroids, etc., are constantly being screened for anticancer activity (Fig. 1 and 2). Some of the active compounds present in this plant are reported to be antioxidant. The activity of compounds should be depended more likely to the type and concentration of cancer cell lines [8]. Denny and Wansbrough [10] reported that the major challenge is to design new drugs that will be more effective for the treatment of cancerous cells and have lesser side effect. Many plant extracts kill and reduce the growth of cancerous cell through activating apoptosis and affecting growth regulators.

However, on the basis review/research articles study, it is revealed that flavonoids, alkaloids, phenolics, tannins, etc., seem to be most likely bioactive compounds that have some anticancer potential.

Table 1: Qualitative biochemical tests result of plant extract

Compounds→	Protein	Alkaloids	Steroids	Phenolics	Flavonoids
Present (+)→	+	+	+	+	+

Table 2: Percentage of cancer cell growth inhibition by plant extract

Tissues→			Liver cancer cell line	Colon cancer cell line
NCFT code	Inst. code	Conc. (µg/ml)	Hep-2	HT-29
NCFT-863	UTU-4	100	82	28
NCFT-868	UTU-9	100	34	**77**
NCFT-869	UTU-10	100	80	68
NCFT-870	UTU-11	100	65	78
NCFT-875	UTU-16	100	72	78
NCFT-876	UTU-17	100	08	78
	Mitomycin-C	1×10^{-6} M	82	80

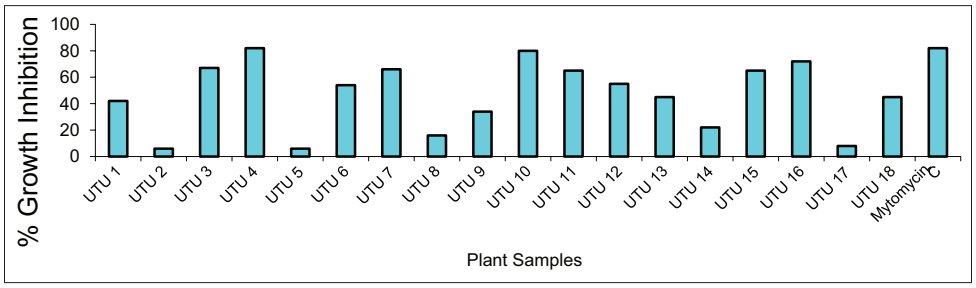

Fig. 1: Percentage of growth inhibition on HEP-2 cell line

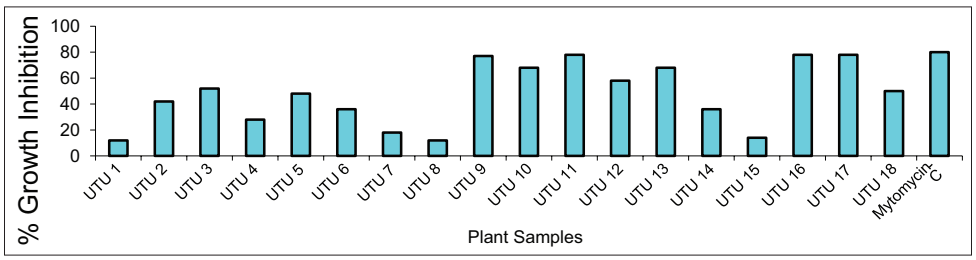

Fig. 2: Percentage of growth inhibition on HT-29 cell line

CONCLUSION

The recent work authenticated the *T. foenum-graecum* plant contains bioactive compounds that have a potential to reduce cancer cell growth *in vitro* by different inhibitory pathways. Many plant extracts destroy cancer cells through activating apoptosis and effecting growth regulators. The research work was very interesting to study the anticancer potential, biochemically identification of compounds, and mechanism of action of the phytoconstituents.

Finally, it can be concluded that the phytoconstituents of this plant can be further used in herbal formulation in pharmaceutics.

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AUTHORS' CONTRIBUTIONS

This recent work was carried out in collaboration by three authors. Author, Shivsharan Singh managed literature searches, designed the study, performed the statistical analysis, and wrote the protocol and first draft of the manuscript. Author, Satish K Verma managed the analysis of the study. Author, Santosh Singh managed the protocol of the study and managed the work done. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

Authors declared that there are no conflicts of interest.

Note: In the present study, ethics approval, consent of patient, and consent for publication are not applicable.

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