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Research Article

IN VITRO CYTOTOXIC AND APOPTOTIC EFFECT OF *PASSIFLORA FOETIDA* AGAINST CERVICAL CANCER CELLS AND ITS FOURIER TRANSFORM INFRARED PROFILING

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ABSTRACT

Objective: This study was aimed on the analysis of cytotoxic and apoptotic action of *Passiflora foetida* followed by identification of the functional groups responsible for the activity.

Methods: In this study, cytotoxic and apoptotic effect of methanol extract of *P. foetida* were analyzed by treating HeLa cell line cultures with different concentrations of the extract (25, 50, 75, 100, and 125 µg/ml), and thereby the activity was ratified by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay and propidium iodide staining. The functional groups of the bioactive compounds for the effectiveness of the treatment were known by Fourier transform infrared spectroscopy analysis (FTIR).

Results: The cytotoxic activity was found to be increased in a dose-dependent manner with inhibitory concentration value of 21.55 µg/ml and showed an effective apoptosis. Further, FTIR analysis confirmed the presence of functional groups of alkaloids, flavonoids, saponins, steroids, terpenoids, phenols and cardiac glycosides which might be responsible for the aforesaid activity.

Conclusion: The cytotoxic and apoptotic action of *P. foetida* was proved to be very effective, and the tenable functional groups were identified.

Keywords: *Passiflora foetida*, Cytotoxic, Apoptotic, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Propidium iodide.

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INTRODUCTION

India is bestowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. Traditional medicine has been practiced for many centuries' especially in rural areas due to its common availability and low cost. The use of medicinal plants for curing diseases had been recorded in the history of all civilizations [1]. The Medicinal plants are considered to be biosynthetic laboratory for its phytochemicals which augment physiological and therapeutic effects [2]. The cancer diseases are fatal to human lives and it is the second leading cause of death in the world. Still no effective medicines are made to treat most cancers [3]. Cervical cancer is found to be the second most common cancer among the women worldwide. Earlier investigation had suggested that infection with human papilloma virus may play a major role in cervical cancer. At present, the treatments available for cervical cancer are mainly synthetic drugs which may induce serious side effects that limit the use of such drugs [4], and also the cancer treatment has become very expensive [5]. Moreover, the anticancer compounds that are available in the market have been isolated from plants in large numbers [6]. Therefore, the use of plants and its products may be the exclusive choice for the prevention and treatment of cancer with lesser side effects and will be an alternative method. At present, the researchers are also focusing greatly on folk medicine to develop better and effective drugs for cancer. Although the properties and doses of medicinal plants are known to the traditional users by practices, still unaware of scientific reasons behind its uses [7,8]. Hence, it will be highly appreciated if more researches are carried out to know the scientific reasons and the actual mechanism of curing diseases either by *in vitro* or *in vivo* experiments. In such a way, this study was aimed by subjecting the commonly used cervical cancer cell line (HeLa cells) to analyze the anticancer effect of widely distributed common medicinal plant, *Passiflora foetida* and to know the functional groups of the bioactive constituent responsible for it through Fourier Transform Infrared (FTIR) profiling. *P. foetida* is an exotic and fast growing perennial vine belongs to the family Passifloraceae reported to have pharmacological properties, *viz*., antimicrobial [9], anti-inflammatory [10], antioxidant [11], antidepressant [12], antidiarrheal and analgesic [13], antinociceptive [14] and antiulcer activities [15]; also used to treat erysipelas and skin diseases with inflammation [16], asthma and biliousness [17], anxiety, insomnia, convulsion, sexual dysfunction, cough and cancer [18]. The other species of the Passifloraceae family such as *Passiflora tetrandra* and *Passiflora edulis* were found to exhibit cytotoxic effect against P-388 murine leukemia cells [19] and CCRF-CEM leukemia cells [20], respectively. Although the report on the anticancer activity of *P. foetida* was available for leaves against breast cancer (MCF 7) cell line [21], fruits against leukemic cell lines (K562, U937, Molt 4, and HL60) [22], the present investigation on cytotoxic and apoptotic potential of methanol extract of *P. foetida* leaves against cervical cancer (HeLa cells) cell line is the first report to the best of our knowledge.

METHODS

Collection of plant sample and preparation of extract

The leaves of *P. foetida* were collected from in and around Erode District, Tamil Nadu, India and taxonomically authenticated by BSI, TNAU, Coimbatore, with reference number BSI/SRC/5/23/2014-15/Tech-1085. The leaves were detached from the healthy collected twigs and then washed thoroughly with tap water followed by distilled water, dried under shadow for about 15 days and then powdered well to extract with methanol according to the earlier described methods of Woisky and Salatino, Cunha *et al*., Phrompittayarat *et al*., and Sasidharan *et al.* [23-26]. The reddish brown precipitate obtained as crude extract was used for the cytotoxic studies and FTIR analysis.

Cell culture

The cytotoxic effect of *P. foetida* was analyzed using HeLa cell line obtained from National Center for Cell Science, Pune, Maharashtra, India, aseptically maintained in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine (1%), streptomycin and penicillin (1%) in a humidified incubator with 5% carbondioxide at 37°C.

Analysis of cytotoxicity by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The methanol extract of *P. foetida* was tested for its cytotoxic effect on HeLa cells and was determined by MTT assay [27]. Cells were seeded at a density of 1×10^5 cells/well in a 96 well plate. The cells were allowed to adhere for 24 hrs and then treated with plant extract at different concentrations (25-125 µg/ml) for 24 hrs. The culture medium was removed and 20 µl of MTT (5 mg/ml in DMEM) was added to each well followed by incubation for 2 hrs to visualize the formazan crystals under a light microscope. The absorbance was measured at a wavelength of 570 nm using a microplate enzyme-linked immunosorbent assay reader after dissolving the formazan crystals in 100 µl isopropanol [28]. The effect of plant extract on HeLa cell proliferation was assessed as percentage cell viability over that of control, where vehicle treated control cells (0.1% dimethyl sulfoxide) were taken as 100% viable. The percentage inhibition of the cytotoxic activity was calculated as follows:

$$\% \text{ Inhibition} = [(\text{Absorbance}_{control}-\text{Absorbance}_{test})/\text{Absorbance}_{control}] \times 100$$

Determination of apoptosis by propidium iodide (PI) staining

The apoptotic action of *P. foetida* on HeLa cells were assessed using fluorescent dye, PI according to the method described [29,30]. HeLa cells 1×10^4 cells/well were seeded in a 24 well plate and grown until confluent. The cells were treated with plant extract at different concentrations (25-125 µg/ml) for 24 hrs. The cells were washed with ice cold phosphate buffered saline (PBS) and fixed with 70% ethanol for 30 minutes. After fixation, the plates were once again rinsed with ice-cold PBS followed by staining with 200 µl of PI (500 µM) for 1 hr; further, the plates were washed twice with ice-cold PBS and the nuclear staining of the cells which undergone apoptosis was observed under a fluorescence microscope.

Statistical analysis

The data obtained for cytotoxicity assay was analyzed using SPSS (16.00) to calculate inhibitory concentration (IC_{50}) value.

FTIR spectrum profiling

The methanol extract of *P. foetida* was mixed with KBr salt using a mortar and pestle thereby compressed into a thin pellet and subjected to record the functional groups responsible for the activity through infrared spectrum on a Shimadzu FTIR spectrometer 8000 series over the frequency ranges between 4000 and 400 cm^{-1}.

RESULTS AND DISCUSSION

Cytotoxic and apoptotic action

The meticulous analysis of cytotoxic and apoptotic action of *P. foetida* (Fig. 1) over HeLa cell line had shown dose-dependent effect in a highly significant manner. The cytotoxic effect was confirmed by percentage of cell inhibition with respect to MTT assay, and the concentration of the methanol extract required to inhibit 50% viability of the cells was determined as IC_{50} value and calculated to be 21.55 µg/ml (Table 1) which revealed the significant toxicity compared to the other medicinal and nonmedicinal plants reported against cervical cancer cells [31,32]. The viable cells with active metabolism converted MTT into a purple colored formazan product which served as a visual marker of the viable cells, and the absorbance measured at 570nm was used to find the IC_{50} of extract over the HeLa cells. The IC_{50} value obtained in this study had proved that *P. foetida* possess an effective cytotoxic effect. As the crude extract possessing an IC_{50} <20 µg/ml are considered to have active compounds against the cancer cells according to the criteria of Standard National Cancer Institute [33,34], the IC_{50} value of this study was found to

Fig. 1: Habit of *Passiflora foetida*

Table 1: Cytotoxic effect of *Passiflora foetida*

Concentration of the extract (µg/ml)	% of inhibition	IC_{50} value (µg/ml)
Control	0	21.55
25	58	
50	62.62	
75	63.66	
100	66.08	
125	74.74	

IC_{50}: Inhibitory concentration

be nearer to the above criteria which strongly recommend and serve as an experimental evidence to utilize *P. foetida* as a natural source of drugs against cancer cells. The uptake of PI was found to increase with increase in the concentration of the extract corresponding to the number of nonviable cells which indicated the apoptotic induction and was clearly visible under fluorescence microscope (Fig. 2). The morphological changes and the characteristics of apoptotic bodies such as blebbing, cell breakage and chromatin condensation were also visualized by PI staining and observed to be similar to the results of the study by Durgha *et al.* [4]. The phytocompounds such as flavonoids, phenolic acids and tannins were found to be the potential sources of anticarcinogenic, anticancer, antimicrobial and antioxidant activities [35,36]. Almost all parts of the plant, *P. foetida* were reported to contain alkaloids, flavonoids, tannins, phenols, steroids, cardioglycosides, saponins and terpenoids [37]. As saponins were found to have membrane permeabilizing property and interfere with the replication of cell DNA, it might have played a role in preventing the growth and division of cancer cells. Furthermore, it was suggested that the phytochemicals in the plants working together with nutrients may slow the aging process and reduce the risk of various diseases including cancer [38,39]. Most of the phytochemicals possess antioxidant potential especially flavonoids which protect the cells against oxidative damage thereby reduces the risk of developing certain types of cancer [40]. The results of this study was analyzed to suspect that the matrix-metalloprotease MMP-2 and MMP-9, enzymes involved in the tumour invasion, metastasis and angiogenesis might also be inhibited by methanol extract prepared from leaves of *P. foetida* as earlier report on such enzyme inhibition was available for aqueous extract of fruits of *P. edulis* and *P. foetida* [41]. Hence, this study had shown the efficacy of *P. foetida* for the cytotoxicity and apoptotic induction toward HeLa cells, thus suggesting the protection against the cervical cancer cells.

FTIR spectrum profiling

The FTIR spectrum recorded for the methanol extract of *P. foetida* had shown various number of peaks as mentioned (Fig. 3). The peak corresponds to functional group of particular phytoconstituent was identified, interpreted and represented (Table 2) according

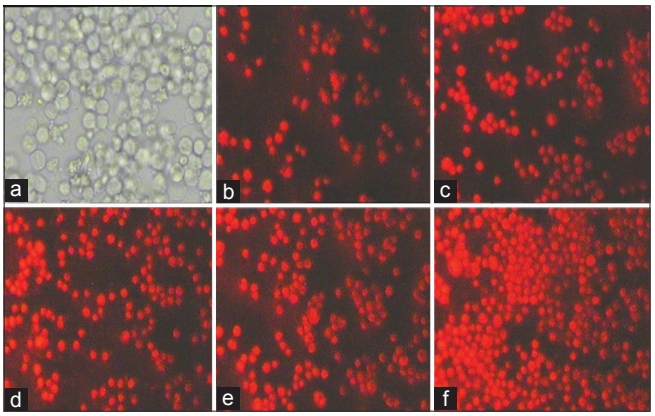

Fig. 2: Apoptotic effect of *Passiflora foetida* on HeLa cells. (a) Controls, (b-f), represent the apoptotic induction of methanol extract at a concentration of 25, 50, 75, 100, and 125 µg/ml, respectively

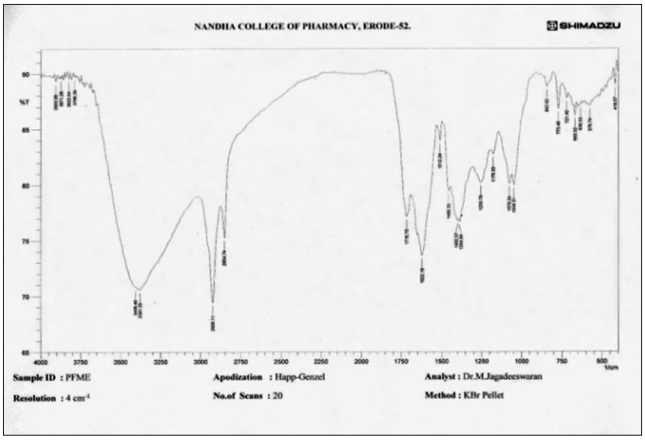

Fig. 3: Fourier transform infrared spectrum of *Passiflora foetida* methanol extract

to the standard reported references [42-48]. The C-O stretching at 1078.24 cm^{-1} revealed the ethers. CH_2 and CH_3 bending peak at 1456.30 cm^{-1} and 721.40 cm^{-1} revealed the aromatic compounds and was identified and found to be similar to the FTIR report of *Aerva lanata* root extract [49] which may strongly support to use the leaves of widely distributed and commonly available *P. foetida* instead of root of *A. lanata* where it is the seasonal and not abundant. The spectral peak at 1716.70 cm^{-1} indicate C=O stretching which may corresponds to acylic or six memberd ketone groups and was found to be the characteristics of saponins as it is the strong and supporting evidence to the earlier report saying that *P. foetida* is rich in saponins [50]. The C-O stretch at 1255.70 cm^{-1} revealed the presence of cardiac glycosides and the strong FTIR spectroscopic finger print peaks at 2854.74 cm^{-1} and 2926.11 cm^{-1} due to C-H stretching corroborated the presence of alkane groups and analyzed to be the characteristics of terpene compounds according to the report of Paulraj *et al.*, [37]. The broad peak at 3406.40 cm^{-1} showed an O-H stretching and indicated the presence of phenol or carbohydrate or alcohol or quinone or carboxylic acid. The presence of quinone may ratify the richness of flavonoids reported to be the major phytoconstituents of *P. foetida* with respect to the previous report and thus it clearly supports the current study [51]. The peak at 3381.33 cm^{-1} indicated the N-H stretch corresponding to primary and secondary amines and found to be the confirmation of alkaloid components. The peak at 1178.5 cm^{-1} and 1384.9 cm^{-1} confirmed the presence of thio group and nitro compounds, respectively. The presence of ketone and alkene groups were revealed by the peak at 1622.19 cm^{-1}. The peak at 1255.70 cm^{-1}, 669.32 cm^{-1}, 576.74 cm^{-1}, 639.53 cm^{-1}, 418.57 cm^{-1}, 773.48 cm^{-1} and 842.92 cm^{-1} were the

Table 2: FTIR peak value and its functional groups

Frequency (cm^{-1})	Component (peaks)	Functional groups
721.4	-CH2 (bend)	Aromatics
1456.3	-CH3 (bend)	
1078.24	C-O stretch	Ethers
1716.7	C=O stretch	Acyclic or six membered ketone group
2854.74	C-H stretch	Alkanes
2926.11		
3406.4	O-H stretch	May be carbohydrate or alcohol or phenol or Quinone or carboxylic acids
3381.33	N-H stretch	Primary and secondary amines
1178.55	-C=S	Thio group
1384.9	N=O	Nitro compounds
1622.19	C=O	Ketones
	C=C	Alkenes
1255.7	C-F/C-O stretch	Halides or Esters or polyol
669.32	C-Br	Halides
576.74		
639.53		
418.57	C-I	
773.48	C-Cl	
842.92		

FTIR: Fourier transform infrared

characteristics of halide groups. Moreover, the C-O stretching peak at 1255.70 cm^{-1} affirmed the presence of esters which were due to the availability of steroids and terpenoids. The presence of phytochemicals analyzed in the methanol extract of *P. foetida* was found to be similar to the methanol leaf extract of *Datura metel* which also had shown the higher anticancer property against MCF-7 cell line [52]. The FTIR spectral profiling of methanolic extract of *Myristica dactyloides* fruit revealed the presence of potential bioactive compounds such as alkaloids, glycosides, flavonoids, and tannins which strongly supports the present work [53]. Thus, the findings of our study had confirmed the presence of functional groups of alkaloids, flavonoids, saponins, steroids, terpenoids, phenols, and cardiac glycosides which might have caused the cytotoxicity and apoptosis.

CONCLUSION

The phytochemicals in plants are having the power and efficacy to cure various diseases and disorders. In such away, this study revealed that the natural source of drugs had acted as a best remedy for the cervical cancer which could be a basement for further analysis against other cancer cells. FTIR analysis ratified the presence of functional groups of the phytocompounds such as alkaloids, flavonoids, saponins, steroids, terpenoids, phenols, and cardiac glycosides with respect to its corresponding peaks. The cytotoxic and apoptotic activity might be enhanced either by individual component or by mixture of compounds that were identified from our investigation in the methanol extract of *P. foetida*. Hence, the actual constituents responsible for curing property can be purified in the near future for the commercialization and betterment of the society to assuage the risk of cancer.

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