Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Identification of Anticancer Compounds from *Linum usitatissimum* seed Extract and their Effect on HeLa Cells

Anitha Joseph, Sriram Sridharan[1], Sampathkumar Palanisamy, Sivakumar Ramalingam, Renuka Saravanan

Department of Chemistry and Biosciences, SASTRA Deemed To Be University, Srinivasa Ramanujan Centre, Kumbakonam, [1]Department of CARISM, SASTRA Deemed to be University, Thanjavur, Tamil Nadu, India

Submitted: 08-Aug-2019 Revised: 26-Sep-2019 Accepted: 15-Feb-2020 Published: 15-Jun-2020

ABSTRACT

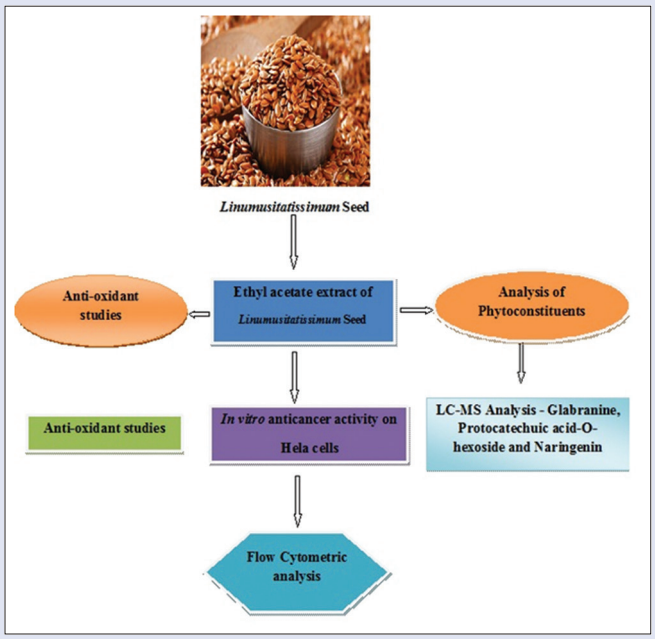
Aim: The aim of the study is to analyze the phytoconstituents and to determine the anticancer potential of *Linum usitatissimum* seed against Human cervical cancer cell line is the correct expansion (HeLa). **Materials and Methods:** The seeds of *L. usitatissimum* were extracted with methanol, ethyl acetate, and chloroform by cold maceration method. These extracts were subjected to qualitative phytochemical analysis, free-radical scavenging ability (1,1-diphenyl-2-picrylhydrazyl) assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and flow cytometric analysis for studying their cytotoxic and apoptotic effect on HeLa cell. Ethyl acetate extract of *L. usitatissimum* seeds was subjected to liquid chromatography–mass spectrometry (LC-MS)/MS analysis for identifying its phytoconstituents. **Results:** Qualitative analysis of the different extracts of the seed revealed the presence of flavonoids, terpenoids, alkaloids, saponins, glycosides, phenols, steroids, and anthraquinones. Free-radical scavenging activity of chloroform extract was found to be more than methanolic and ethyl acetate extracts. Further, methanol, ethyl acetate, and chloroform extracts exerted cytotoxic effect with an 50% inhibitory concentration value of 21 µg/ml, 17.5 µg/ml, and 20 µg/ml, respectively. Flow cytometric analysis revealed that the number of proapoptotic and late apoptotic cells were found to increase with the increasing concentration of seed extract. LC-MS/MS analysis of ethyl acetate extract revealed the presence of glabranine, protocatechuic acid-O-hexoside, and naringenin. **Conclusion:** The study demonstrated the anticancer effect of the seeds of *L. usitatissimum*, and the presence of glabranine and naringenin, well-known anticancer agents, is being reported for the first time in *L. usitatissimum* seed. The antioxidant and anticancer potential of the seed could be due to the presence of glabranine and naringenin.

Key words: Anticancer, antioxidant, HeLa, *Linum usitatissimum*, liquid chromatography–mass spectrometry/mass spectrometry

SUMMARY

- The present study explored the anticancer effect of *Linum usitatissimum* seed extract and the results obtained have been promising and could be used as preliminary data for further detailed studies on this seed extract against cancer.

Abbreviations used: Hela: Human Cervical carcinoma; NCCS: National Center for Cell Sciences; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DPPH: 1,1-diphenyl-2-picrylhydrazyl; LC-MS: Liquid chromatography–mass spectrometry; BSS: Balanced Salt Solution; DMSO: Dimethyl sulfoxide; FITC: Fluorescein isothiocyanate; TIC: Total Ion Chromatogram.



Correspondence:

Dr. Renuka Saravanan,
Department of Chemistry and Biosciences, SASTRA Deemed to be University, Srinivasa Ramanujan Centre, Kumbakonam - 612 001, Tamil Nadu, India.
E-mail: renuka@src.sastra.edu

DOI: 10.4103/pm.pm_341_19

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Cervical cancer is a malignant neoplasm that arises from the uterine cervix, and the lesions are a significant risk to women's health. Cervical cancer ranks as the second major female cancer[1] and it is one of the foremost causes of mortality and morbidity throughout the world under the age of 45.[2]

Around 500,000 women have been universally diagnosed with cervical cancer and approximately 280,000 deaths occur yearly, of which 80% of cases occur in developing countries, like India. India has the most considerable burden of cervical cancer patients in the world. One woman dies of cervical cancer every 8 min in India.[3]

Cite this article as: Joseph A, Sridharan S, Palanisamy S, Ramalingam S, Saravanan R. Identification of anticancer compounds from *Linum usitatissimum* seed extract and their effect on HeLa cells. Phcog Mag 2020;16:221-6.

Treatment includes single therapy or combination therapy such as radiotherapy, hysterectomy, surgery, pelvic exenteration, chemotherapy, pelvic lymph node dissection, biological techniques, trachelectomy, chemoradiation, and conization.[4] Despite high treatment benefits, conventional therapies exhibit toxicity against normal cells and are also associated with severe side effects.

On the other hand, biological-based therapies include the use of herbs; dietary supplements as well as traditional medicine systems which are now attracting global attention as potential sources of anticancer agents. They are extensively used because of their availability, applicability, affordability, therapeutic efficacy, and with little or no side effects, which, in turn, has accelerated the scientific research on these agents.[5]

Isolation of compounds through bioactivity is a prominent strategy for the discovery of effective anticancer agents.[6] Plants have proved to be a new source of effective anticancer materials. Recently, many prominent anticancer drugs such as analogs, etoposide analogs, camptothecin, indicine-N-oxide, Taxol, topotecan, vinblastine, and vincristine are all derived from natural products.[7,8]

There are also reports existing on medicinal plants such as *Aesculus indica*, *Garcinia mangostana*, *Pfaffia paniculata*, *Sapium ellipticum*, *Solanum nigrum*, *Artemisia vulgaris*, and *Vernonia amygdalina*[6] that relieve and treat various cancers by utilizing phytocompounds that occur naturally with antioxidant and anticancer properties and are known to inhibit or kill the carcinogenic cells.[9] Furthermore, it has been reported that several secondary metabolites isolated from the medicinal plants can decrease cell proliferation, retard metastasis, inhibit angiogenesis, and induce apoptosis.[10]

L. usitatissimum also called as common flax or linseed is a minor oilseed belonging to the family *Linaceae* and it is one of the world's oldest arable crops. It is used in the treatment of high cholesterol, heart disease, menopausal symptoms, ulcers, and type 2 diabetes. Several epidemiological reports are available on the potential anticancer effect of *L. usitatissimum* seed against prostate, breast, and colon cancer cells. The present study showcases the remarkable anticancer potential of different extracts of *L. usitatissimum* seed against the human cervical cancer cell line (HeLa) through various *in vitro* assays.

MATERIALS AND METHODS

Collection of seeds

The seeds of *L. usitatissimum* were collected from in and around Kumbakonam, Tamil Nadu, India, during 2018. The seeds were shade dried for 10 days and were powdered with an electronic blender. It was stored in an airtight container at room temperature till use.

Preparation of seed extracts

About 50 g of the seed powder was macerated with 300 ml of methanol, ethyl acetate, and chloroform for 72 h in a sterile condition. The extract obtained was filtered and evaporated using a boiling water bath for 30 min at 60°C.

Preliminary phytochemical screening

Preliminary Phytochemical screening of *L. usitatissimum* seed was carried out using standard textual procedures and screened mainly for the presence of carbohydrates, alkaloids, flavonoids, quinones, and triterpenoids.[11]

Test for alkaloids

About 0.5 g of crude leaf powder was diluted with 2 N hydrochloric acid and distilled water. The mixture was heated over a water bath for a few minutes, cooled, and filtered. The filtrate was tested by adding alkaloid specific reagents such as Mayer, Wagner, and Dragendorff.

Test for steroid/triterpenoids

The crude leaf powder was macerated with 20 ml of hexane for 2 h and then filtered. The filtrate was evaporated in the evaporating dish and then added few drops of Liebermann–Burchard reagent. The onset of blue or blue–green color denoted the presence of steroid, the appearance of red, pink, or purple indicated the presence of triterpenoids.

Test for flavonoids

The crude leaf powder was shaken with hot water for 5 min and filtered under warm conditions; magnesium powder, concentrated hydrochloric acid, and amyl alcohol were added to the filtrate and shaken. The development of red or yellow or orange color indicated the presence of flavonoids.

Test for carbohydrates

The crude leaf powder was mixed with the α-naphthol solution in alcohol and then added few drops of sulfuric acid along the sides of test tubes. The appearance of a violet ring at the junction of two layers confirmed the presence of carbohydrates.

Test for quinones

The crude leaf powder was mixed with 2 ml of 10% NaOH solution. The onset of red color indicated the presence of quinones.

In vitro antioxidant activity

Radical Scavenging activity of 1,1-diphenyl-2-picrylhydrazyl

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was performed by a standard method.[12] DPPH was used to measure the free-radical scavenging activity of the extract. About 0.3Mm solution of DPPH in 95% methanol was prepared. One milliliter of this solution was added to 3 ml of the fraction dissolved in chloroform, methanol, and ethyl acetate at various concentrations and allowed to stand in a dark room at room temperature for 30 min. The absorbance was recorded at 515 nm in a colorimeter and the experiment was repeated thrice. The decrease in absorbance of the DPPH solution indicated an increase in antioxidant activity. The free-radical scavenging activity was expressed as a percentage of inhibition of the DPPH radical. The antioxidant activity was expressed as:

% of disappearance = (control − sample)/(control) × 100

Cell line and culture conditions

HeLa (human cervical carcinoma) cell line was obtained from the National Center for Cell Science, Pune, India. The cells were cultured in minimum essential medium (MEM) medium (MEM + fetal calf serum) supplemented with 2 mM l-glutamine and balanced salt solution adjusted to contain 1.5 g/L Na_2CO_3, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 2 mM l-glutamine, 1.5 g/L glucose, 10 mM (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), and 10% fetal bovine serum (GIBCO, USA). Penicillin and streptomycin (100 IU/100 μg) were adjusted to 1 mL/L. The cells were maintained at 37°C with 5% CO_2 in a humidified CO_2 incubator.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide assay

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a colorimetric assay used to evaluate the cell viability. Cytotoxic assay on HeLa cell line was performed by a standard method.[13] The cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h into 85% confluence. The medium was replaced with fresh medium containing the serially diluted compound and the cells were further incubated for 48 h. The

culture medium was removed and 100 µL of the MTT (Hi-Media) solution was added to each well and incubated at 37C for 4 h. After removal of the supernatant, 50 µL of dimethyl sulfoxide was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm using an ELISA multiwell plate reader (Thermo Multiskan EX, USA). The OD value was used to calculate the percentage of viability using the following formula,

% of viability = (OD value of the experimental sample)/(OD value of the experimental control) ×100

The effects of extracts were expressed by the 50% inhibitory concentration (IC_{50}) values. The IC_{50} was defined as the concentration that reduced the absorbance of the treated cells by 50% concerning untreated cells.

Apoptotic analysis by flow cytometer

Flow cytometry is a multiparametric tool that allows the measurement of many apoptosis characteristics in one single sample. Apoptosis was determined by double staining with fluorescein isothiocyanate (FITC) conjugated Annexin V as described previously.[14] Briefly, HeLa cells (1×10^5) were seeded in a 6-well plate. After 24-h incubation at 37°C (5% CO_2), the medium was changed with fresh, supplemented, or not (control) with the complex (50 and 100 µM). After 24-h incubation, cells were harvested with trypsin, washed with phosphate-buffered saline (PBS), fixed in 70% ethanol, and stored at −20°C for 1 h. Then, the cells were stained with Annexin-V/FITC, followed by removing the ethanol, and washed with PBS; the cells were suspended in 0.5 ml PBS containing Annexin-V/FITC µg/ml, 100 µg/ml RNase and incubated at 37°C for 30 min. Flow cytometry was performed in duplicate with a BD FACS flow cytometer.

Liquid chromatography–mass spectrometry/mass spectrometry

Identification of active principles in the ethyl acetate extract of the plant sample was done through liquid chromatography–mass spectrometry (LC-MS)/MS analysis (C_{18} RP Acclaim 120Å, 2.1 mm × 150 mm, 3 µm Column, Ultra High-Performance Liquid Chromatography (UHPLC) Dionex and micrOTOF-Q II, Bruker Daltonics). LC was carried out at a flow rate of 0.2 mL min^{-1} and wavelength 280 nm. A discontinuous gradient starting with 100% B (MilliQ water + 0.1% formic acid) from 0 to 9 min, 80% B from 10 to 14 min, 70% B from 15 to 24 min, 50% B from 25to 34 min, 25% B from 35 to 44 min, 0% B at 45 min, and 100% B at 47 min was followed. 100% B was maintained for another 5 min till the end of the run. Mobile Phase A comprised acetonitrile + 0.1% formic acid. The molecular weight of the identified secondary metabolites was obtained through Electrospray Ionization (ESI) MS carried out in negative mode with optimized parameters of N_2 flow rate at 6.0 L/min, 24.7 psi nebulizer pressure, 280°C capillary temperature, capillary voltage at 3.5KV and a scan range of 50–1000 m/z.[15]

RESULTS

Table 1 represents the preliminary phytochemical screening results of various extracts of *L. usitatissimum* seed. The methanol extract was found to contain flavonoids, saponins, terpenoids, glycosides, alkaloids, steroids, and anthraquinones. Flavonoids, terpenoids, glycosides, alkaloids, steroids, phenols, and anthraquinones were identified in the extract of ethyl acetate. The chloroform extract showed the presence of flavonoids, steroids, terpenoids, alkaloids, saponins, glycosides, and anthraquinones. Tannin was completely absent in all three extracts.

Figure 1 represents the DPPH antioxidant activity of methanol, ethyl acetate, and chloroform extracts of *L. usitatissimum* seeds. The results revealed that the chloroform extract of *L. usitatissimum* seed showed an increased scavenging activity when compared with ethyl acetate and methanol extracts. The scavenging activity of DPPH radical by these extracts was compared with the standard (ascorbic acid).

Antioxidant activity of chloroform, ethyl acetate, and methanol extracts of *L. usitatissimum* seed was found to increase with increasing concentration of the extract. Thus, the present results of the DPPH assay showed that the seeds of *L. usitatissimum* have potent antioxidant property.

Table 1: Preliminary phytochemical screening of the various extracts of the *Linum usitatissimum* seeds

Phytoconstituents	Methanol extract	Ethyl acetate extract	Chloroform extract
Alkaloids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+
Saponins	+	-	-
Tannins	-	-	-
Glycosides	+	+	+
Phenols	-	+	-
Anthraquinones	+	+	+
Protein	+	+	+
Carbohydrates	+	+	+

+: Presence; −: Absence

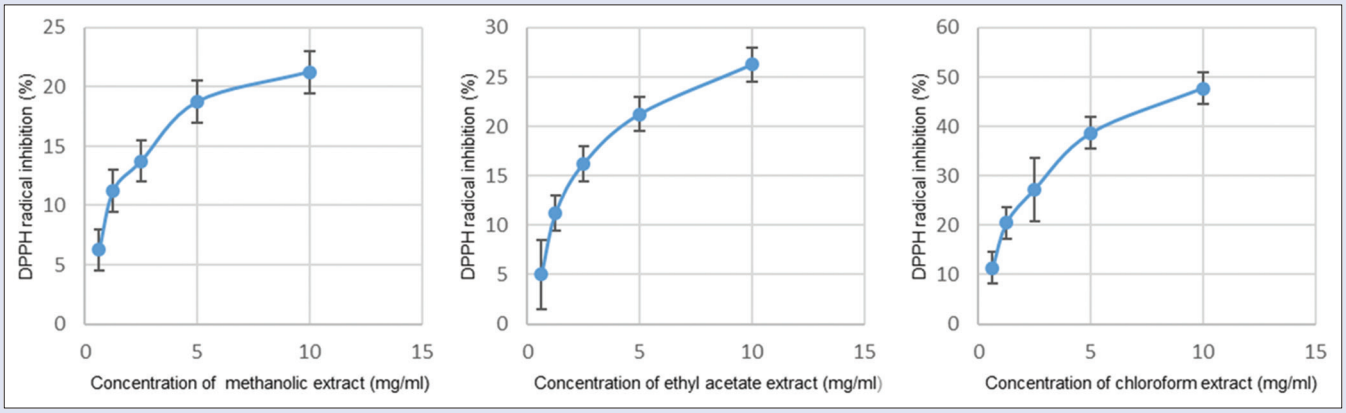


Figure 1: 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity of different extract of *Linum usitatissimum* seeds

Figure 2 represents the cytotoxic effect of chloroform, ethyl acetate, and methanol extracts of *L. usitatissimum* seed against human cervical cancer cell line (HeLa). From the result, it was observed that the inhibitory activity (IC_{50}) for methanol, ethyl acetate, and chloroform was found to be 21 ± 1.5 µg/ml, 17.5 ± 0.5 µg/ml, and 20 ± 0.5 µg/ml, respectively. Of these, ethyl acetate extract showed marked cytotoxic activity against HeLa cell line.

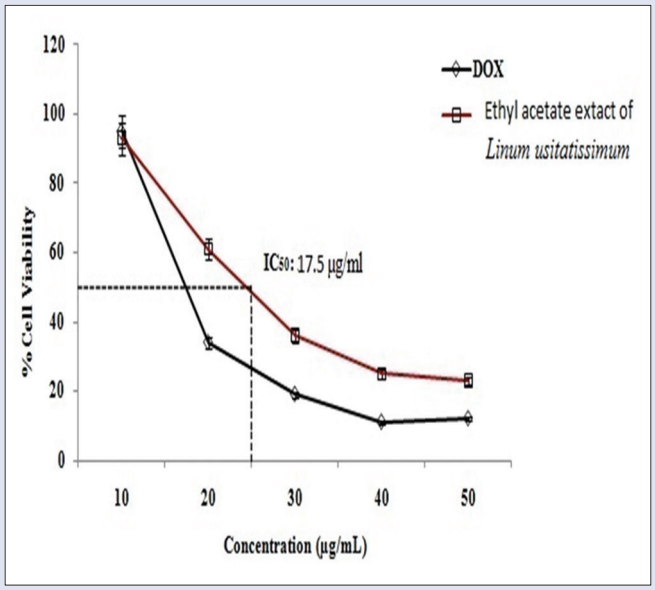


Figure 2: Cytotoxic effect of different extracts of *Linum usitatissimum* seed on HeLa cell line

In the current study, we found that *L. usitatissimum* seed showed cancer cell-specific toxicity compared to normal cells after 24 h.

Figure 3 shows the flow cytometric analysis of apoptotic detection on the HeLa cell line. Cells treated with no extract (control) showed no late and proapoptotic cells, whereas cells treated with increasing concentration of plant extract showed more proapoptotic and late apoptotic cells. Treatment with 60 µg/ml of ethyl acetate extract of *L. usitatissimum* seed exhibited a significant population of pro and late apoptotic cells in the right-hand quadrants of flow cytometric graphs. These results indicate that the dose-dependent ethyl acetate extract of *L. usitatissimum* induced cell death facilitated by the induction of apoptotic pathways in HeLa cells more significantly.

Screening of phytoconstituents in ethyl acetate extract of *L. usitatissimum* seeds led to the identification of a complex mixture of flavonoids and phenols predominantly containing glabranine, protocatechuic acid-o-hexoside, and naringenin in LC-MS/MS analysis. The total ion chromatogram (TIC) of the ethyl acetate extract of *L. usitatissimum* seeds is represented in Figure 4. The retention time and mass of the compounds identified along with their respective fragmented peaks are listed in Table 2.

Flavonoid derivatives present in the plant were identified using m/z values and MS/MS pattern obtained in the TIC and by comparing them

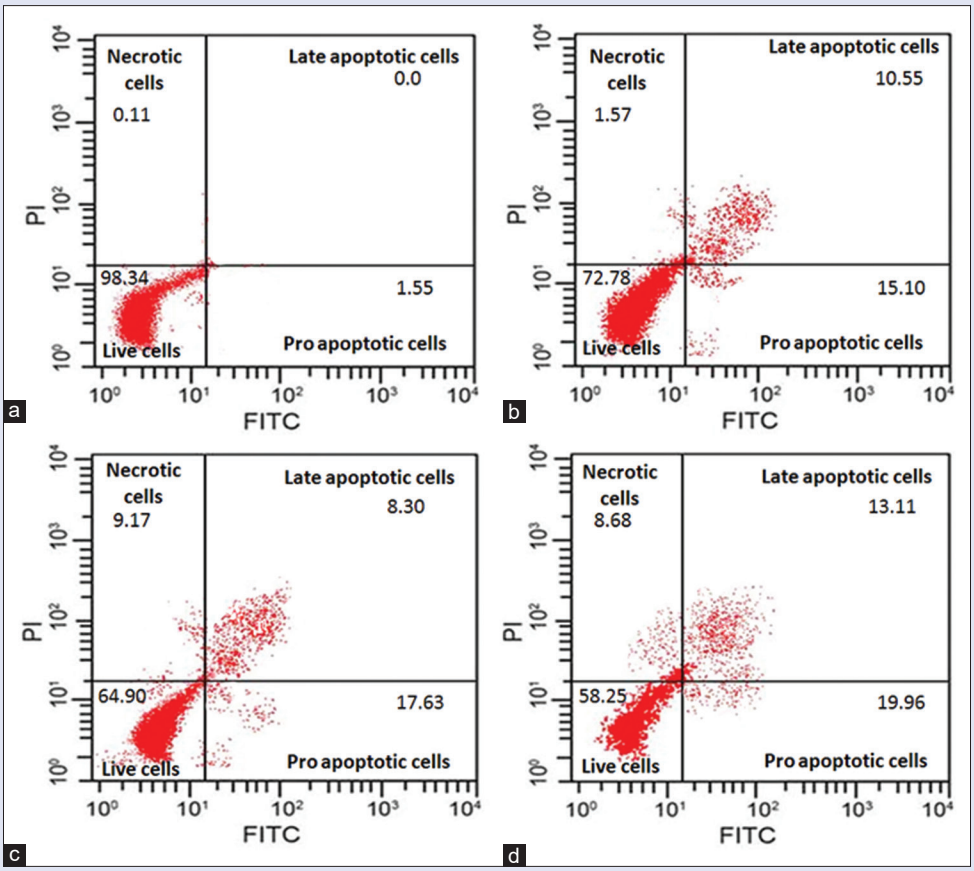


Figure 3: Flowcytometric analysis of apoptotic detection on HeLa cell line. Quadrant 4 represents necrotic cells, quadrant 3 represents late apoptotic cells, quadrant 2 represents proapoptotic cells, and quadrant 1 represents live cells. (a) Control, (b) 20µg/ml treated HeLa cells, (c) 30µg/ml treated HeLa cells, and (d) 60µg/ml treated HeLa cells

to those of the molecules available in various online databases. Figure 5a, 5b, and 5c represents the MS/MS pattern of identified flavonoids and its derivatives.

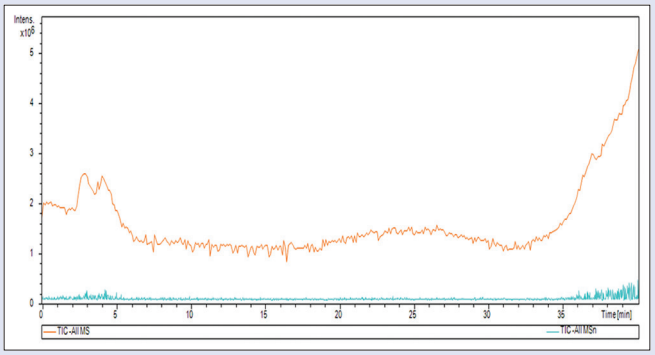


Figure 4: Total ion chromatogram of ethyl acetate extract of *Linum usitatissimum* seeds

DISCUSSION

Development of cancer is a complex process mediated by different stimuli, which, in turn, leads to cellular and molecular damage. Free radicals play a significant role in the development of different types of cancer, as they alter the DNA and turn the normal cell into a cancerous cell, one of the mechanisms of carcinogenesis. Therefore, it is essential to determine the free-radical scavenging effect of an anticancer therapeutic agent (*in vitro*). Secondary metabolites have a varied spectrum of therapeutic effect such as anticancer, antiviral, and anti-inflammatory properties and also exhibit antioxidant and free-radical scavenging activities. In this study, we have reported the presence of several phytoconstituents with proven anticancer effect in *L. usitatissimum* seed extract. The result obtained in this study is in accordance with a previous study[16] which observed a dose-dependent free-radical scavenging effect of *Thymelaea hirstuta* extract using ascorbic acid as standard.

The relative amount of live and dead HeLa cells was determined by flow cytometric analysis which further supports the apoptotic effect of the seed extract of *L. usitatissimum*. The ethyl acetate extract of the seed

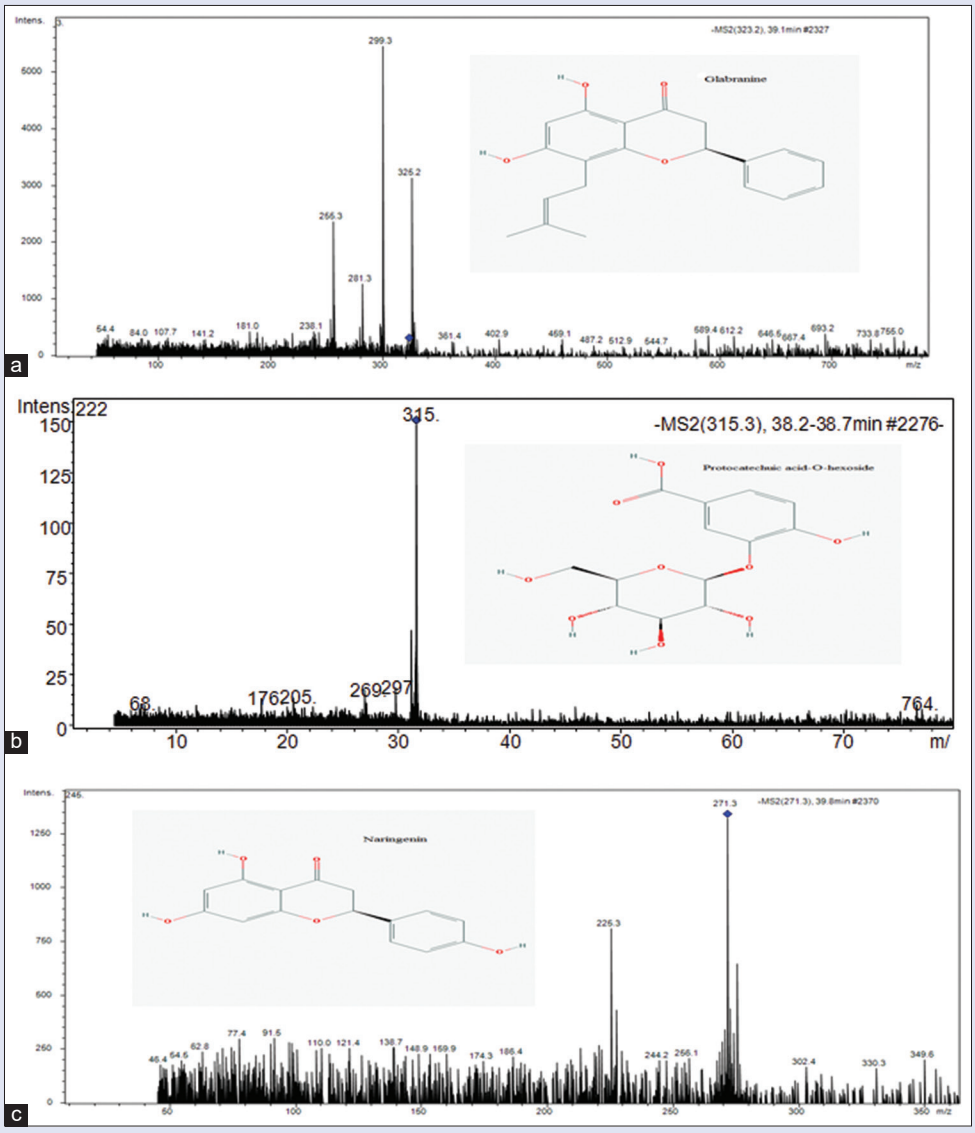


Figure 5: liquid chromatography–mass spectrometry/mass spectrometry compounds identified in the ethyl acetate extract of *Linum usitatissimum* seeds (a) glabranine (b) protocatechuic acid-O-hexoside (c) naringenin

Table 2: Electrospray ionization-mass spectrometry/mass spectrometry product ions of ethyl acetate extract of *Linum usitatissimum* seed

Identified compound	Rt (min)	Mass	Parent ion [M-H]-	MSMS (product ion)	Reference
Glabranine	39.1	324	323	107, 141, 181, 219, 238, 255, 279, 281,	Mass Bank database (BML00154)
Protocatechuic acid-O-hexoside	38.2-38.7	316	315	269, 223	V. Spínola *et al.*/Food Chemistry 173 (2015) 14-30
Naringenin	39.8	272	271	119, 151, 169, 177, 227, 271, 273	Mass Bank database (FIO00211)

showed a dose-dependent apoptotic effect against HeLa cells, which is in line with a previous finding.[17] The apoptotic pathway could either be through the intrinsic or extrinsic pathway. The exact mechanism of induction of apoptosis for the extract or the compounds is not known and yet to be explored. Naringenin, a flavone that has been identified in this study, has been reported to possess anti-carcinogenic effect,[18] which induces apoptosis through G_0/G_1 phase arrest, caspase activation, and DNA fragmentation.[19]

Similarly, glabranine, a flavanone, has also been reported to possess anticancer effect against MiaPaCa-2 cell line[20] and is also documented for its antiviral activity.[21] Protocatechuic acid-o-hexoside was also reported for its antitumor effect in animal models.[22] MTT assay is a well-established method to assess the cytotoxic effect of compounds against different cells and is based on the ability of Nicotinamide adenine dinucleotide (NADH)-dependent cellular oxidoreductase enzymes to reduce MTT and the reduction is proportional to the number of viable cells, a tool to measure cell viability. We observed a decreased reduction of MTT in HeLa cells, which might be the reason to induce the apoptosis in a dose-dependent manner of the seed extracts, and hence, the plant extract could be used as an anticancer and antioxidant agent. A similar report[23] showed the cytotoxic effect of *Ganoderma* against different cancer cells.

CONCLUSION

The data obtained through *in vitro* studies indicate that the *L. usitatissimum* seed extract possesses significant anticancer and antioxidant activities. Phytocompounds such as glabranine, protocatechuic acid-o-hexoside, and naringenin detected in the LC-MS/MS analysis further provided evidence for the anticancer action of the selected extract. Furthermore, the presence of glabranine and naringenin in the *L. usitatissimum* seed extract is being reported for the first time in the present study.

Acknowledgements

The authors are thankful to the Department of Chemistry and Biosciences, SASTRA Deemed to be University, Srinivasa Ramanujan Center, Kumbakonam.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ramesh E, Alshatwi AA. Naringin induces death receptor and mitochondria-mediated apoptosis in human cervical cancer (SiHa) cells. Food Chem Toxicol 2013;51:97-105.
2. Rajendra AK, Yogesh VK. Screening for cervical cancer: An overview. J ObstetGynecol India 2006;56:115-22.
3. Uma P, Pooja B. What is cervical cancer? J Gynecol Women's Health 2017;2:1-4.
4. van de Vooren K, Curto A, Garattini L. Curing cervical cancer or preventing it: A case of opportunity cost in the long run? Vaccine 2014;32:6867-9.
5. Sonika J, Jaya D, Pankaj KJ, Swaha S, Arjun P. Medicinal plants for treatment of cancer: A brief review. Pharmacogn J 2016;8:87-102.
6. Renuka S, Brindha P, Sivakumar R. A review on the role of phytoconstituents in breast cancer cells. Int J PharmTech Res 2014;6:799-808.
7. Shaikh AM, Shrivastava B, Apte KG, Navale SD. Medicinal plants as potential source of anticancer agents: A review. J Pharmacogn Phytochem 2016;5:291-5.
8. Renuka S, Brindha P, Mahesh N, Sivakumar R. Gas chromatography-mass spectrometry analysis, *in vitro* cytotoxic and antioxidant efficacy studies on *Cleome gynandra L.* (leaves): A traditional drug sourc. Asian J Pharm Clin Res 2017;10:84-9.
9. Lakshmi PM, Bhanu PK, Venkata SK, Josthna P. Herbal and medicinal plants molecules towards the treatment of cancer: A mini review. Am J Ethnomed 2015;2:136-42.
10. Kashafi E, Moradzadeh M, Mohamadkhani A, Erfanian S. Kaempferol increases apoptosis in human cervical cancer HeLa cells via PI3K/AKT and telomerase pathways. Biomed Pharmacother 2017;89:573-7.
11. Harbone JB. Phytochemical Methods, a Guide to Modern Techniques of Plant Analysis. London: Fakenham Press; 1973. p. 33-80.
12. Gyamfi MA, Aniya Y. Antioxidant properties of thonningianin a, isolated from the African medicinal herb, *Thonningia sanguinea*. Biochem Pharmacol 2002;63:1725-37.7.
13. Ramar T, Palani G, Krishnasamy K, Gnanaiah S, Shenbagamoorthy S, Manickam P, *et al.* A novel disintegrin protein from Najanaja venom induces cytotoxicity and apoptosis in human cancer cell lines *in vitro*. Process Biochem 2012;47:1243-9.
14. Krishnamoorthy K, Sundaravadivelu S, Manickam P, Palakkad RP. Increased anticancer efficacy by the combined administration of quercetin in multidrug resistant breast cancer cells. BMR Cancer Res 2014;1:1-3.
15. Arun PK, Rajesh SS, Sundaram SM, Sivaraman T, Pemiah B. Structural characterizations of lead anti-cancer compounds from the methanolic extract of *Jatropha tanjorensis*. Bangladesh J Pharmacol 2014;9:452-65.
16. Hulya T, Faik AA, Nesrin C, Jiri G, Miroslav S. Phenolic acid content and free radical scavenging activity of two differently processed carob tree (*Ceratonia siliqua L.*) pod. Food Nutri Sci 2013;4:547-53.
17. Kavitha CV, Nambiar M, Ananda Kumar CS, Choudhary B, Muniyappa K, Rangappa KS, *et al.* Novel derivatives of spirohydantoin induce growth inhibition followed by apoptosis in leukemia cells. Biochem Pharmacol 2009;77:348-63.
18. Bao L, Liu F, Guo HB, Li Y, Tan BB, Zhang WX, *et al.* Naringenin inhibits proliferation, migration, and invasion as well as induces apoptosis of gastric cancer SGC7901 cell line by downregulation of AKT pathway. Tumour Biol 2016;37:11365-74.
19. Ahamad MS, Siddiqui S, Jafri A, Ahmad S, Afzal M, Arshad M. Induction of apoptosis and antiproliferative activity of naringenin in human epidermoid carcinoma cell through ROS generation and cell cycle arrest. PLoS One 2014;9:e110003.
20. Andrade-Carrera B, Clares B, Noé V, Mallandrich M, Calpena AC, García ML, *et al.* Cytotoxic evaluation of (2S)-5,7-dihydroxy-6-prenylflavanone derivatives loaded PLGA nanoparticles against MiaPaCa-2 Cells. Molecules 2017;22. pii: E1553.
21. Abd Kadir SL, Yaakob H, Mohamed Zulkifli R. Potential anti-dengue medicinal plants: A review. J Nat Med 2013;67:677-89.
22. Hong C, Xue MW, Xue LZ. Anti-thrombotic and anti-tumor effects of water extract of caulis of *Sargentodoxa cuneata* (Oliv) Rehd et Wils (*Lardiza balaceae*) in animal models. Trop J Pharm Res 2016;15:2391-6.
23. Faruck LH, Mohammed AB, Jackson A. Chemical composition and anti-proliferative effect of Oman's *Ganoderma applanatum* on breast cancer and cervical cancer cells. J Taibah Univ Med Sci 2016;11:145-51. 23.