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ANTIMICROBIAL, RADICAL SCAVENGING AND CYTOTOXICITY STUDIES OF SAPONIN RICH PLANTS

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ABSTRACT

Present study aims to determine antimicrobial and antioxidant activities of crude methanolic extract and cytotoxicity activity of saponin extracts of T. foenum-graecum (Fenugreek) and S. indicum (Seasame -Black and White) seeds on human breast cancer cell line (MCF-7). The antimicrobial activity was determined by Agar Cup Diffusion assay against three common bacteria S. aureus, S. typhimurium and E. faecalis and a fungus C. albicans. Methanolic extracts of all three seeds were found to be effective on all test microorganisms. Both Fenugreek and White sesame extracts have shown maximum zone of inhibition of 1.7 cm on S. typhimurium while only Fenugreek seed extract has shown zone of inhibition of 1.6 cm on S. aureus. Antioxidant activity was determined using DPPH and ABTS free-radical scavenging assays taking BHT (Butylated hydroxytoluene) as positive control. Methanolic extract of Fenugreek seeds has shown IC_{50} value at lowest concentration of 130µg/mL for ABTS assay in comparison to positive control which was found to be 65µg/mL. The cytotoxic activity of the pure Saponin extracts was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Sulforhodamine B (SRB) and Lactate dehydrogenase (LDH) assays. Amongst different assays highest IC_{50} concentration was obtained in White sesame extract by MTT assay (20 µg/ml), in Black sesame extract by LDH (26.87 µg/ml) and SRB assay (10.0 µg/ml) in comparison to positive control Doxorubicin IC_{50} value varying from 7.0 to 9.5 µg/ml concentrations. Results showed that Saponin extract of Fenugreek and Sesame seeds significantly reduced the viability of cancerous cells though not in dose-dependent manner. It can be concluded from present study that methanolic crude and Saponin rich extracts of seeds of T. foenum-graecum (Fenugreek) and S. indicum (Seasame) can be a potential source of antimicrobial, antioxidant and anticancer natural products.

KEY WORDS

Antioxidant, Antimicrobial, Cytotoxicity, MCF-7 cell line, T. foenum-graecum, S. indicum.

INTRODUCTION

All over the world, scientists are exploring medicinal plants for finding out pharmacologically active compounds. Screening of medicinal plants for their phytochemical, antimicrobial, antioxidant and anticancer activities is of prime concern[1,2]. The assessment of the antioxidant, anticancer and antimicrobial activities of traditional medicinal plants serve as the baseline for the chemical identification of active molecules or lead compounds which could be chemically manipulated into effective drugs[3]. Saponins are naturally occurring surface-active glycosides. They derive their name from their ability to form stable, soap-like foams in aqueous solutions. This easily observable character has attracted human interest from ancient times. Saponins are common in a variety of higher plants and usually found in roots, tubers, leaves, blooms or seeds. Based on the carbon skeletons, saponins were classified into triterpenes and steroids. Saponins occur constitutively in many plants species, both wild plants as well as cultivated crops. In cultivated crops triterpenoid saponins are generally predominant, while steroid saponins are common in plants used as herbs or for their health-promoting properties[4]. Many saponins are known to be antimicrobial inhibit mould and protect plants from insect attack. *Trigonella foenum-graecum* (commonly known as Fenugreek) belongs to family Fabaceae. Fenugreek is the small stony seeds from the

pod of a bean-like plant. The seeds are hard, yellowish brown and angular. Some are oblong, some rhombic, other virtually cubic, with a size of about 3mm (1/8"). Diosgenin, a steroid sapogenin is found in fenugreek including other Sapogenins: Yamogenin, Gitogenin, Tigogenin, and Neotigogen. *Sesamum indicum* L. (Commonly known as Sesame), a member of Pedaliaceae family, is an annual shrub with white bell-shaped flowers. It is grown for seeds that are rich in oil content. Sesame seeds are about 3 to 4 mm long by 2 mm wide and 1 mm thick. The seeds are ovate, slightly flattened, and somewhat thinner at the eye of the seed than at the opposite end. Sesame seeds occur in many colors depending on the cultivar. Sesame has compounds like sesamin, sesaminol, gamma tocopherol, cephalin and lecithin. These compounds impart many of the pharmacological activities like antioxidant, antibacterial, cardiotonic, antidiabetic, hypocholesterolemic, antitumor, antiulcer, anti-inflammatory and analgesic properties[5]. The aim of present study is to determine antimicrobial and antioxidant activities of crude methanolic extract and cytotoxicity activity of saponin extracts of T. *foenum-graecum (*Fenugreek) and *S. indicum* (Seasame -Black and White) seeds on human breast cancer cell line (MCF-7).

MATERIALS AND METHODS

Collection of Plant materials

The seeds of *Trigonella foenum-graecum* L. commonly known as Fenugreek or Methi and *Sesamum indicum* L. also known as Sesame or Til (Black and White Sesame seeds) were bought from Hakeem Chichi Pharmacy, Rani Talao, Surat, Gujarat. The seeds were dried overnight in oven at 60˚C. After drying, they were grounded well into fine powder and stored in air tight container.

Procurement of Test Microorganisms

The test microorganisms for the study included fungus-*Candida albicans* (NCIM No. 3628), gram positive *Staphylococcus aureus* (NCIM No. 5345) and *Enterococcus faecalis* (NCIM No. 5252) and *Salmonella typhimurium* (NCIM No. 5278). Test organisms were maintained on appropriate maintenance medium i.e. Nutrient agar for *S. typhimurium* and *S. aureus* at 37˚C, MRS medium for *E. faecalis* at 37˚C and MGYP medium for *C. albicans* at 28˚C.

Procurement of MCF-7 Breast Cancer Cell line

MCF-7 (Human Breast Adeno carcinoma cell line) was procured from National Centre for Cell Sciences (NCCS), Pune, India. Cell line was maintained with regular subculturing at the interval of 3 days using Minimum Essential Medium (MEM) complete medium supplemented with 10% Fetal Bovine Serum (FBS) and Penicilin (100U)-Streptomycin (100 µg/ml) antibiotics. The cells were then subjected to *in-vitro* analysis.

Preparation of Aqueous extract: 5 gm of powder was suspended in 20 ml of distilled water and subjected to constant shaking in water bath at 50˚C for 4-5 hours. The mixture was filtered using muslin cloth and filtrate was dried under low pressure. A solution containing 20 mg/ml of extract was prepared in distilled water for analyses.

Preparation of Organic (methanolic) extract by Soxhlet extraction: 10 gm of dried seed powder was packed in a thimble separately and extracted in Soxhlet extractor using 150 ml of methanol (40°C) until a clear sample was obtained (7-8 cycles). At the end of extractions, the extracts were dried at 50-60˚C on hot plate. The residue was dissolved in absolute methanol and 15% DMSO solution to obtain 20 mg/ml stocks of crude seed extract and stored in sterile container at 4 ˚C in refrigerator until use.

Determination of Saponins

Isolation of saponins was performed using Obadoni and Ochuko (2001)[6] protocol. 20 g of each sample powder was placed into a conical flask and 100 ml of 20% aqueous ethanol were added to it. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered, and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight and the saponin content was calculated as percentage.

Saponin (%) = {Final weight of sample /Initial weight of extracts} X 100**Qualitative Analysis of Saponins**

Froth test: Froth test was performed to detect the presence of saponins in extracts. 0.5 ml of sample was added in 10 ml of distilled water in a test tube and shaken vigorously for about 30 seconds. It was allowed to stand for 30 min. and the height of honeycomb froth produced was observed and recorded.

Thin Layer Chromatography: The qualitative analysis using Thin Layer Chromatography plays an important role in the study of saponins. For detection of saponins, the seed saponin fractions (saponin fraction, aqueous fraction and NaCl fraction) were spotted manually using a capillary tube on heat activated and precoated silica gel 60 G TLC plates 20X20 cm plates, layer thickness 250 µm (Merck KgaA, Darmstadt, Germany). The spotted plates were put into n-butanol: glacial acetic acid: water = 4:1:5 solvent system. After the separation the plates were sprayed with p-anisaldehyde solution and heated for 10 min at 110^0C. The colour of the spots was noted and the movement of the analyze was expressed by its retention factor (R*f*). R_f values were calculated using the formula:

Retention time (R_f)=

Distance travelled by solute/Distance travelled by solvent

Antimicrobial Studies

The antimicrobial assay was performed by Agar Well Diffusion method. The organisms tested along with the positive controls were mentioned in Table 1. 20 ml of medium was poured into the petri plates and was left to solidify. To the solidified medium 100 µl of 10^5 CFU/ml cells were added and was uniformly spread using the glass spreader. Six wells were prepared in the agar plate with the help of a sterile cup-borer (0.8 cm). Into the 3 wells, 100 µl of the plant extracts (crude methanolic extract, aqueous extract and saponin fraction of ethanolic extract) prepared in 15% DMSO were introduced. In 2 wells, 15% DMSO and distilled water were added (negative control) and in opposite well 100 µl of antibiotic (positive control) was introduced. The plates were then incubated overnight at appropriate temperature. Antimicrobial activity was determined by measuring the diameter of the zone of the inhibition [7].

Table 1- Micro-organisms tested for antimicrobial study

Organism	Nutrient medium	Incubation temperature	Positive control
C. albicans	MGYP medium	28˚ C	Fluconazole
E. faecalis	MRS medium	37˚C	Ciprofloxacin
S. aureus	Nutrient agar	37˚C	Streptomycin
S. typhii	Nutrient agar	37˚C	Streptomycin

***In vitro* Antioxidant assays**

ABTS radical scavenging assay:

ABTS (2, 2-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) assay measures the relative stability of antioxidant to scavenge the ABTS generated in aqueous phase, as compared with the standard (ascorbic acid or BHT). ABTS assay was performed according to Re *et al* (1999)[8]. The stock solution was prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM potassium persulfate solution followed by incubation for 16 h at room temperature in the dark to yield a dark-colored solution containing ABTS•+ radicals. Working solution was prepared freshly before each assay by diluting the stock solution by mixing of stock solution to 50% methanol for an initial absorbance of about 0.700 (±0.02) at 745 nm, with temperature control set at 30°C. Free radical scavenging activity was assessed by mixing 300 µl of different fractions (25-500 µg/ml for standard BHT and 25, 50, 100 and 200 µg/ml for plant extracts) with 3.0 ml of ABTS working standard. The decrease in absorbance was measured after mixing the solution. Data for each assay was recorded in triplicate. BHT was used as positive control.

DPPH radical scavenging assay:

DPPH (1,1-diphenyl-2-picrylhydrazyl) is considered as a stable radical because of delocalization of spare electron over the molecule. DPPH assay was performed as described by Re *et al.* The free-radical scavenging activity of BHT and different plant extracts was measured with the stable radical diphenyl picrylhydrazyl (DPPH) in terms of hydrogen-donating or radical-scavenging activity. 3 ml of DPPH (in methanol) solution having 0.980 (± 0.02) absorbance was added to 100 µl of different fractions, at different concentrations (25-500 µg/ml for standard BHT and 25, 50, 100 and 200 µg/ml of plant extracts). Tubes were incubated for 30 min in dark. After 30 min, the absorbance was measured at 517 nm. Control was prepared by adding 100µl methanol and 3 ml diluted DPPH. Lower absorbance of the reaction mixture indicates higher free radical

scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}, which was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%. BHT was used as a positive control. Each study corresponded to three experiments, performed in duplicate.

The scavenging activities of both DPPH and ABTS radicals were estimated by the following formula:

% Scavenging = $[(A_0 - A_s)/A_0] \times 100$

Where: A_0 is absorption of control; A_S is absorption of tested extract solution.

***In vitro* Studies**

Cell line Maintenance: MCF-7 Breast Cancer Cells serve as an excellent *in vitro* model for studying the mechanism of tumor response. The medium was brought to room temperature by thawing. The tissue culture bottles were observed for growth, cell degeneration, pH and turbidity in inverted microscope. After the cells became 80% confluent sub culturing was done. The mouth of the bottle was wiped off by using spirit-soaked cotton to remove the adhering particles. The breast cancer (MCF-7) cells were cultured in Minimal Essential Media (MEM) supplemented with 10% Fetal Bovine Serum (FBS), (100U) 20µg/ml penicillin and 100µg/ml streptomycin antibiotic. Incubation was carried out at 37°C with an atmosphere of 5% CO_2.

Dilution of drugs: The stock solution of anticancer drug Doxorubicin having final concentration 1 mg/ml was prepared in serum free MEM media. The IC_{50} of doxorubicin was found to be 10 µg/ml, so stock was further diluted to different concentrations ranging from 5, 10 to 15 µg/ml. Stock solutions having final concentration 1 mg/ml of saponin extracts of *Trigonella foenum-graecum* and *Sesamum indicum* were prepared in serum-free MEM medium. The samples were syringe filtered using 0.22 µM membrane filter to remove contaminants. Range of dilutions (6.25, 12.50 and 25.00 µg/ml) of extracts was prepared in sterile conditions in laminar flow hood by adding calculated amounts of MEM to the stock solution.

***In vitro* cytotoxicity assays**

Cell-based assays are often used for screening collection of compounds to determine if the test molecules have effects on cell proliferation or show direct cytotoxic effects that eventually lead to cell death. There are variety of assay methods that can be used to estimate the number of viable Eukaryotic cells. The methods described include: MTT, SRB and LDH.

MTT assay: This is based on the quantitative measurement of extracellular reduction of the tetrazolium dye 3-[4,5-dimethylethiazole -2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) to insoluble formazan crystals by metabolically active cells. MTT solution (HiMedia) was prepared at concentration of 5 mg/ml in PBS (Phosphate Buffered Saline solution) as stock solution. Briefly, $5x10^4$ cells were transferred into 96-well microtitre plates containing 100 µl of medium and incubated for 24 h at 37˚C in a 5% CO_2. The culture medium was replaced by 100 µl of various concentrations of plant extract (drug) i.e 6.25, 12.50 and 25.00 µg/ml, and incubated for 24h. Then, 10µl MTT reagent was added in each well and incubated for 4h. After incubation, 100µl of solubilization solution was added to each well and absorbance was read on ELISA reader at 490 nm. Assay was performed in triplicates. The IC_{50} values were calculated by plotting OD readings versus the drug concentrations.

LDH assay: Lactate Dehydrogenase *(*LDH*)* is an oxidoreductase, which is soluble cytosolic enzyme present in most eukaryotic cells*.* ***Procedure was carried out according to LDH HiMedia kit protocol.*** MCF-7 cells were seeded in 96-well plate (5×10^3 cells/well) in medium containing 10% FBS and incubated for 24 h under 5% CO_2 at 37°C for attachment. Then the cells were washed with PBS, 100 µL of the plant extract *(*drug*)* in a concentration of 6.25, 12.5, 25 µg/ml were added to each test well in triplicates and incubated for 24 h. After incubation, 50µl supernatant from each well was transferred to a new microtitre plate containing 50µl LDH reagent and re-incubated for 2h. Reaction was stopped by adding 50µl stop solution and absorbance was measured at 490 nm. % cytotoxicity was calculated and IC_{50} value was determined by plotting OD versus drug concentration.

SRB assay: The Sulphorhdamine B assay is used for determination of cell proliferation and cytotoxicity based on the measurement of cellular protein content**.** Procedure was carried out according to SRB HiMedia kit protocol. 100 µl cell suspension with density 5×10^3 to 10^4 per well were seeded in 96-well plate**.** After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed once and 100µl of plant extract *(*drug*)* in a concentration of 6.25, 12.5, 25 µg/ml were added to the cells in microtitre plate. The plate was then incubated at 37°C for 24h. After incubation, 25µl of cold fixative-50% trichloroacetic acid

was added to the wells gently such that it forms a thin layer over the test compounds and plate was incubated at 4°C for 2h. The plates were flicked and washed 3 times with 100µl washing solution to remove traces of medium, sample and serum, and were then air dried. The air-dried plates were stained with 50µl SRB and kept for 30 minutes at room temperature. The unbound dye was removed by rapidly washing 3 times with 25µl washing solution. The plates were then air-dried. 100µl of solubilization solution was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate reader at a wavelength of 490 nm**.** The IC_{50} values were calculated by plotting OD readings versus the drug concentrations.

RESULTS AND DISCUSSION

Saponin Determination

Saponins were determined by Obadoni & Ochuko method. The residue was dried and dissolved in PBS. The highest percentage of saponin content was obtained in Fenugreek extract (7.96) while the lowest in Black sesame (0.56). White sesame seed extract was found to have 1.13% of saponin content. Presence of saponins and polyphenols had been shown by previous studies in both Fenugreek and Seasame seeds[10,11].

Qualitative Analysis

Froth test: Froth test was conducted to detect the presence of saponins in the crude extract as well as saponin fractions. Standard Saponin powder was used as positive control for froth test. Appeciable level of frothing was observed in ethanolic Saponin fraction while it was found absent in aqueous and methanolic crude extracts.

Thin layer Chromatography: Different fractions of Fenugreek and Sesame seeds were subjected to TLC and solvent system n-butanol: Glacial Acetic acid: Water (4:1:5) was used for the detection of saponins after optimization of serial solvent systems. Blue, violet, pink and yellow spots were seen after the derivatization with anisaldehyde sulphuric acid reagent. Based on the analysis of the chromatogram, it can be stated that as there are differences in R_f values, different types of saponins are present in different plant species used in present study (Table 2).

Table 2- R_f values of standard and Saponin fractions of Fenugreek, Black Sesame and White Sesame seed extract by TLC

Sr. no.	Fenugreek		Black Sesame		White Sesame	
	Std. Saponin	Saponin fraction	Std. Saponin	Saponin fraction	Std. Saponin	Saponin fraction
1	0.375	0.437	0.321	0.345	0.35	0.350
2	0.437	0.475	0.357	0.452	0.425	0.487
3	0.475	0.675	0.404	0.500	0.500	0.612
4	0.500	0.75	0.440	0.547	0.575	0.662
5	0.662		0.714		0.712	
6	0.762					

Antimicrobial studies

The antimicrobial assay of the methanolic, aqueous and Saponin extracts against selected microorganisms was carried out by the agar cup diffusion assay and results are shown in Table 3. All three extracts had shown antimicrobial activity against commonly occurring disease causing bacteria however the zone of inhibition diameter was less compared to the positive controls. This depicts that these extracts can be a source of antimicrobial compounds utilized against these bacterial species.

Table 3- Inhibition zone diameter by agar cup diffusion assay

Microorganism	Plant extract	Zone of inhibition in diameter (cm); D of cup borer= 0.8 cm			
		Methanolic extract	Aqueous extract	Saponin extract	Positive control
C. albicans	Fenugreek	1.1	1.2	0.8	1.5
	B Sesame	1.1	1.0	1.1	1.7
	W Sesame	1.1	1.1	1.3	1.7
E. faecalis	Fenugreek	1.0	0.8	0.9	1.3
	B Sesame	1.1	1.0	1.0	1.5
	W Sesame	1.0	0.8	1.1	1.6
S. aureus	Fenugreek	1.6	1.5	1.4	2.3
	B Sesame	1.5	1.3	1.5	2.1
	W Sesame	0.8	1.5	1.4	2.5
S. typhimurium	Fenugreek	1.7	1.5	1.4	2.3
	B Sesame	1.5	1.5	1.3	2.0
	W Sesame	1.7	1.3	1.0	2.4

***In vitro* Antioxidant assays**

Two most commonly used *in vitro* antioxidant assays DPPH and ABTS were used to determine the antioxidant potential of the methanolic extracts of Fenugreek and Sesame seeds. Concentrations of the extracts used in present study were 25, 50, 100 and 200µg/ml. Results obtained were shown in Table 4 & 5 and Figure 1. Maximium percentage inhibition was shown by Fenugreek extract by ABTS assay at 200µg/ml concentration while lowest by White Seasame seeds. With DPPH radical scavenging assay percentage inhibition was very low in comparison to ABTS assay. Differences in percentage of inhibition would be due to difference in principle on which these two methods work. Previous studies had shown that methanolic Fenugreek extract possessed radical scavenging activity and found that higher the amount of the phenolic compounds and reducing power, higher the percent ABTS and DPPH radical scavenging activity[12]. The antioxidant potential of the extracts measured by ABTS and DPPH scavenging method was also expressed as 50% inhibitory concentration, IC_{50} values. The IC_{50} value of methanolic extract of Fenugreek, Black Sesame and White Sesame seeds had free radical scavenging capacity (IC_{50} >250 µg/mL) close to that of the positive control BHT in the DPPH assay. The Fenugreek extract also had high activity (IC_{50} of 130µg/mL) as assessed by ABTS assay. The results in Table 6 underline that different radicals have different antioxidant potentials (this mean kinetics) when reacting with phenolic compounds. The molecular size of both the radicals is different. ABTS radical has to be formed initially, while DPPH is really a stabilized radical by itself. Furthermore, they might have also different affinities against other compounds that are present in the samples. Certain bioactives compounds may not be soluble into reaction media and cannot express radical scavenging activities. ABTS is better and more sensitive assay as it can be used to detemine the antioxidant potential of a large range of samples (Both hydrophillic and lipophillic substances). Also, DPPH assay cannot be used for those substances which have an overlapping spectrum of absorbance similar to that of DPPH itself.

Table 4- Radical scavenging of plant extracts by ABTS assay

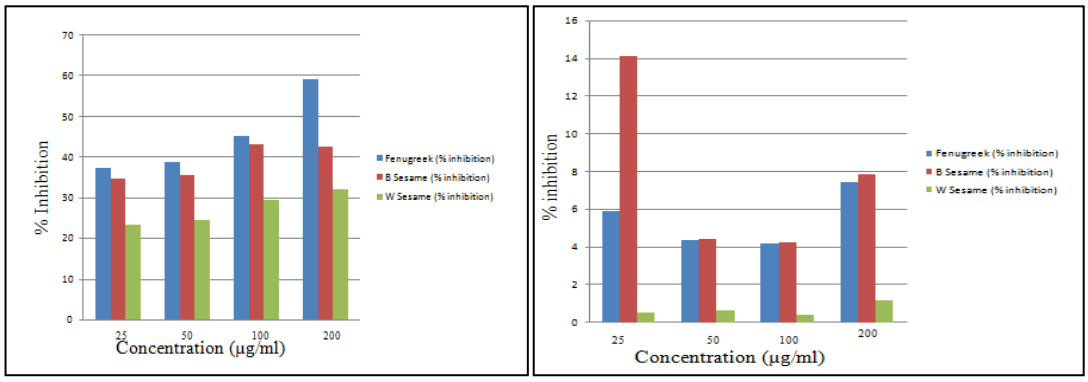
Concentration (µg/ml)	Fenugreek (%Inhibition)	B Sesame (%Inhibition)	W Sesame (%Inhibition)
25	37.37	34.64	23.20
50	38.73	35.49	24.40
100	45.22	43.17	29.35
200	59.21	42.49	32.08

Table 5- Radical scavenging of plant extracts by DPPH assay

Concentration (µg/ml)	Fenugreek (% Inhibition)	B Sesame (% Inhibition)	W Sesame (% Inhibition)
25	5.93	14.13	0.52
50	4.34	4.44	0.63
100	4.17	4.23	0.42
200	7.41	7.83	1.16

Table 6-IC_{50} values obtained by radical scavenging assays

Extracts	IC_{50} value by ABTS assay (µg/ml)	IC_{50} value by DPPH assay (µg/ml)
BHT	65	252.25
Fenugreek	130	>250
Black Sesame	175	>250
White Sesame	250	>250



(a) (b)

Figure 1- Graphical representation of antioxidant potential of different concentration of methanolic plant extracts using (a) ABTS and (b) DPPH radical scavenging assays.

***In vitro* Cytotoxicity Studies**

The cytotoxic activity of Saponin extracts of *Trigonella foenum-graecum*, *Sesamum indicum* (Black & white seeds) and standard anticancer drug Doxorubicin was assessed against Human Breast Adenocarcinoma Cell line (MCF-7) using following cytotoxicity assays *viz.* MTT, LDH and SRB. The results in Figure 2 showed that the MCF-7 cells treated with different concentration of seed significantly reduced the viability of cancer cells. 6.25µg/ml concentration of white sesame extract showed highest cytotoxicity (72.48%) by MTT assay while 12.5µg/ml had shown 82.81% by LDH assay. 25 µg/ml concentration of Fenugreek extract showed highest cytotoxicity (86.13%) by SRB assay. The average % cytotoxicity of 10 µg/ml concentration of Doxorubicin was 60.19%. The results showed that the saponin extract of Fenugreek and Sesame seeds significantly reduced the viability of cancer cells but not in a dose-dependent manner. Amongst different assays highest IC_{50} concentration was obtained in white sesame extract by MTT assay (20 µg/ml), in black sesame extract (26.87 µg/ml) and (10.0 µg/ml) by LDH and SRB assays respectively. It had been reported that Fenugreek seed extract significantly inhibited 7,12-dimethylbenz (a) anthracene-induced mammary hyperplasia and reduces its incidence in rats and advised that the anti-breast cancer protective effects of Fenugreek could be due to increased apoptosis[13]. Another research group had examined the effect of ethanolic extract of Fenugreek on MCF-7 and reported that it had decreased the cell viability and induced early apoptotic changes[14]. Sesamin from Sesame extract had been reported to reduce cell viability by inducing necrosis and apoptosis in MCF 7 cells by LDH release and TUNEL assays [15]. Same compound Sesamin also caused cell cycle arrest at G1 phase in human breast cancer MCF 7 cells[16]. In present study, the reduction in percent cell viability after 24 h of treatment showed potent cytotoxic effects of

Fenugreek and Sesame extracts on MCF 7 Breast cancer cell line.

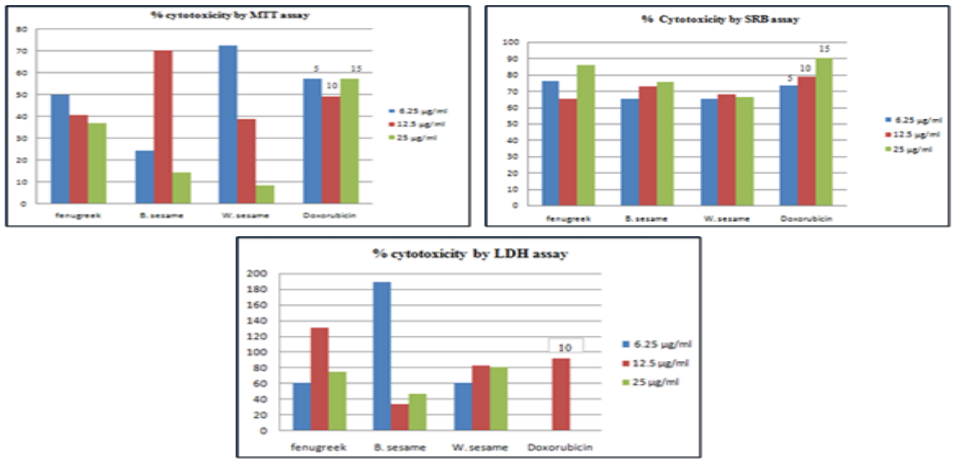


Figure 2- Graphs showing results of various Cytotoxicity assays

CONCLUSION

Plants used in traditional medicines have been accepted as leads for therapeutic drug development in modern medicine. The extracts of Fenugreek and Sesame seed selected for present study was seeds are commonly used in food preparations due to their strong flavour and aroma. The seeds are reported to have restorative and nutritive properties, such as anti-diabetic activity, hepatoprotective activity, antioxidant properties and anticancer potential against experimental cataract. Both these seeds were reported to have high content of Saponins and saponin rich seeds were experimentally shown protection against breast and colon cancers [5]. Hence preliminary studies performed on saponin extracts of *T. foenum-graecum* and *S. indicum*n had shown to possess different level of anticancer, antimicrobial and antioxidant properties, which is of importance for the development of new therapeutic agents against breast cancer. Further research is required to be done to know about the molecular mechanism underlying the apoptotic action on cancer cells as well as to identify the specific compound with its biological property.

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