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Original Article

Anti-cancer and Anti-oxidant Potencies of *Cuscuta reflexa* Roxb. Plant Extracts

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ABSTRACT

All around the world, the percentage of deaths due to cancer is continuously increasing-the greatest devastation of deaths. Among all medications, Unani medicines are boon for human beings to treat cancer with no or least side effects. About 80% rural population use natural products for primary health care. *Cuscuta reflexa* Roxb. (family Cuscutaceae) is utilized in traditional medicines for curing cancer and other diseases, and it is considered as the most significant plant in the Unani medicinal system. The extracts of *Cuscuta reflexa* Roxb. were obtained to measure the anticancer activity with H-1299 and MCF-7 cancer cell lines. Soxhlet extraction was utilized for stem and seeds. The anticancer activity of fractions of each extract obtained by using Flash chromatography was also checked. Besides, the antioxidant activity of each fraction was also checked. DNA binding study supported the results obtained during whole process. The cellular death was detected utilizing ELISA. The results indicated that extracts of *Cuscuta reflexa* Roxb. exhibited strong anticancer activities as compared to the fractions of each extract. *Cuscuta reflexa* Roxb. extracts indicated noteworthy cytotoxicity against human H-1299 and (lung cancer) MCF-7 cancer cells (breast cancer). The extract of this plant may be given to the patients having lung cancer and breast cancer.

Keywords: *Cuscuta reflexa* Roxb., H-1299, MCF-7 cell lines, Cytotoxicity, Anticancer treatment

INTRODUCTION

Today, there are many diseases around us, but cancer is the riskiest ailment [1-12]. The rates of death due to cancer are similar in males as well as in female [13]. All over the world, the different treatments for cancer are being used. For chemotherapy, some drugs have been synthesized chemically and used in cancer treatment, which has many side effects [14-17]. But among all the treatments, Unani medicines have their own unique position in cancer therapy [18]. In Asia, there are different types of plants having bioactive compounds, which have been used as Unani medicines. Aftimoon with botanical name *Cuscuta reflexa* Roxb. is one of them [19]. This plant is parasitic in nature with a slender yellow stem as it draws nutrition from the host after fixing on other plant's body [20]. It is widely found in temperate and tropical regions and commonly found throughout India. In Ayurvedic medicine, aftimoon has been used in various diseases such as diseases of eye, heart, digestive system, diaphoretic, protracted fever, purgative etc.[21-27]. The chemical examinations have been carried out along with pharmacological studies on this plant. The seeds and stems of aftimoon are of high medicinal values for carminative, anodyne, purgative, hair growth etc.[28,29]. The seeds are given as a depuration and carmination in stomach pains and aches. The seed poultice can also be used locally for removing pains. The stems in decoction are useful in flatulence, constipation, liver complaints and bilious affection [30]. Equiguard is a Chinses medicine for curing kidney problems and aftimoon is one of the constituents of this. Research of New York Medical College has indicated that the use of Equiguard may be beneficial to treat cancer [31]. It may be due to apoptosis of cancer cells; describing the therapeutically aspects of this plant [32]. The authors reported the use of the plant in the treatment of melanous,

melancholia, numpness, epilepsy, facial palsy, paralysis, worm infestation, arthritis, weakness of liver, jaundice, stomach and spleen. Furthermore, the authors also described the plant applications in solving the problems of the resolvent, cathartic to black bile and phlegm, anti-helmintic demulcent, and carminative. Besides, *In vitro* study on the plants indicated their antioxidant activity *via* free radicals scavenging activity due to phenolic compounds in their extracts.[33] Chatterjee et al. [25] reported the antitumor activity of *Cuscuta reflexa roxb.* With swiss albino mice using hot water extract of *Cuscuta reflexa roxb.* The extract was applied on the skin papillomas and carcinomas induced by 7,12-dimethylbenz[a]anthracene. In this way, aftimoon is having quite good anti-cancer activities and, hence, it is considered as the most important plant in the Unani system of medicine but unfortunately, no systematic study is carried out. In view of this, the systematic study of this plant is carried out to determine the anticancer potential. The plant material is extracted with different solvents, and fractionated with flash chromatography. The different fractions were studied through DNA binding, antioxidant and anticancer activities. Finally, the chemical compositions of the most active extracts and fractions were determined (Figure 1).

METHOD

The following work was carried out under this section.

Collection and Authenticity of Plant

The plant materials (of 3 months old; plant age) were collected from Deoband (Saharanpur) of U.P., India and the genus and species of the medicinal plants are authenticated through Indian Agriculture Research Institute, Delhi, India, where specimen for plant was deposited.

Preparation of Plant Extracts

The extractions of the plant stems and seed were carried out in the different solvents using Soxhlet apparatus. The collected plant materials were dried in the shed and powdered using the grinder. 25 g Powder of both dried stems and seeds were taken in a Soxhlet apparatus with 500 mL of extracting solvents. The plant stems and seeds were extracted in various solvents i.e. plant stem in acetone (I), plant stem in acetonitrile (II), plant stem in ethyl acetate (III), plant stem in methanol (MeOH; IV), plant stem in water-methanol (H_2O-MeOH; 50:50; V), plant stem in water (H_2O; VI), plant seed in methanol (MeOH; VII) and plant seed in water (H_2O; VIII), separately and respectively. During the extraction procedure, the colorless extracting solvents became colored indicating the dissolution of the plant material constituents in the solvents. The extraction was carried out till the extracting solvents became colorless in the rotary evaporator apparatus arm. After this procedure, the extracts were filtered using Whatman filter paper No. 24. The filtrates were reduced to 50 mL using a vacuum rotary evaporator apparatus. These reduced filtrates were kept in a freeze for further studies.

Anticancer Activities

The anticancer activities of the plant extracts were assessed by performing cell line studies by colorimetric assays (MTT and MTS both). Tetrazolium dyes are the most versatile dyes used for cytotoxicity (loss of viable cells) or cytostatic activity (proliferative to resting status shift) studies. XTT [(2,3-bis-(2-Methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide); $C_{22}H_{17}N_7O_{13}S_2$], MTS and WSTS (water-soluble tetrazolium salts) are the most studied dyes for cell line studies. MTT is widely used for assessment of cytotoxicity, cell viability and proliferation studies in cell biology. MTT gives a yellowish aqueous solution, which on reduction by mitochondrial succinate dehydrogenase, present in metabolically active cells, yields a water-insoluble violet-blue formazan crystal. The lipid-soluble formazan product may be extracted with organic solvents (e.g. DMSO, iso-propanol; C_2H_6OS, C_3H_8O) and estimated by ELISA reader in the range of 500-600 nm. Eight plant extracts were tested for their cytotoxic activities against human breast cancer cell line (MCF-7) and lung cancer cell line (H-1299) at different concentrations using MTT assay according to the method of Mosmann. The anticancer activities of the plants' extracts were performed with different concentrations such as 200, 150, 100 and 50 µg/mL on two different cancer cell lines i.e. H-1299 (lung cancer) and MCF-7 (breast cancer) cell lines. *In vitro* anticancer profiles of the plant extracts were screened against human lung cancer (H-1299) and breast cancer cell lines (MCF-7) by MTT assay. DMEM (low glucose), 10% fetal bovine serum and antibiotics/antimycotics formed the main constituents of the culture medium. The cancer cell lines were seeded in 96-well plate at a density of 2×10^3 cells/well. These were incubated at 37°C under a humidified atmosphere containing 5% CO_2 for 24 h before assay. The cells were further incubated in media containing various concentrations of the extracts. After 24 h, the medium was removed and washed with PBS. About 20 µL of MTT solution was added to each well followed by 4 h incubation at 37°C. Subsequently, the medium was removed followed by addition of 200 µl DMSO. After slowly shaking (twice) for 5 s, the absorbance of each well was determined at 570 nm. The cell viability (%) was calculated as the ratio of the number of surviving cells with test compounds and blank. Similarly, the anticancer studies on lung and breast cancer cell lines were performed using the MTS; developed by Promega. The compounds and the extracts were dissolved in DMSO at a concentration of 0.5 mg mL^{-1}. The investigation was carried out by dilution of stock solution in the ratio of 1:10, 1:100, 1:1000 and 1:10000. The samples of cells, grown in non-modified medium, served as controls. After 24 h of incubation, MTS colorimetric assay of cell survival was performed. The wells were treated with MTS solution and incubated for 2 h at 37° C under 5% carbon dioxide and 95% air atmosphere. The absorbance of each well at 490

nm was read by an automatic microplate reader (''Tecan'', Austria). Relative cell viability, expressed as a percentage of the untreated control (100% viability), was calculated for each concentration.

Fractionation of Plant Extracts

After checking the anticancer potential of *Cuscuta reflexa* Roxb. extracts, fractionation of these extracts was carried out by flash chromatography; to find out the number of components and their activities. The flash chromatography with a binary and quaternary solvent delivery system, UV-Vis/Dual variable wavelength detector, Cheeta purification software (Bonna-Agela Technology, China.) was used. Hexane-Ethyl Acetate-MeOH of varied ratio was used as the mobile phase with flow rate of 30 mL/minute. The column used was of silica (20 g, 40-60 μm). 500 mg Crude extract was mixed with silica to make it in the form of slurry and loaded into silica column and eluted with the above-mentioned solvent in increasing polarity ratio. The fractions (30 mL) were obtained with an increase in polarity of the mobile phase i.e. with the increasing volume of ethyl acetate. The purity of each fraction was checked by TLC using methanol-chloroform (1:9, v/v) as a mobile phase. The spots were detected and identified in a long UV cabinet. Besides, TLC plates were also identified using iodine chamber. The fractions having the same R_f were pooled together to obtain the individual fraction. After fractionation, the anticancer activity of each fraction was also checked.

DNA Binding Studies

Literature indicates that about 90% anticancer agents act by attacking DNA of the cells [34-39]. Therefore, the attempts were made to study the bindings/interactions of the plant extracts with DNA. The study of interactions of the plant extracts with C_t-DNA was carried out at pH 7.4 in a solution of double distilled water containing tris-(hydroxymethyl)-amino methane (Tris, 10^{-2} M). Initially, the concentration of freshly prepared C_t-DNA solution was determined by UV–Vis absorption spectrophotometry at a wavelength of 260 nm (ε = 6600 M^{-1} cm^{-1}) [40]. The purity of DNA as a stock solution of C_t-DNA was determined by taking the ratio of A260/A280 ≥ 1.80, which indicates the sufficiently protein-free nature of DNA [41]. The concentration of the stock solution of DNA was experimentally determined by utilizing ε=6600 M^{-1} cm^{-1} at 260 nm. The additional solutions of DNA were made from a stock solution (30 × 10^{-4} M) with a fixed concentrated solution of plant extracts (0.01 mg/mL).

To carry binding experiments, the absorption spectra of freshly prepared plant extracts at a fixed concentration of (0.01 mg/mL) were taken with the increase in the concentration of DNA (1.0 × 10^{-5} to 30.0 × 10^{-4} M). First of all, λ_{max} and absorbance of pure DNA and compounds were recorded separately in tris-buffer solutions. Then λ_{max} and absorbance of mixture i.e. 2.0 mL of each solution of DNA and compounds were recorded. The absorption spectra were recorded after each addition of the different concentrations of DNA solution (2.0 mL). To produce constant results, the titration experiments were repeated five times. The intrinsic binding constants (K_b) were determined by Benssi-Hilderbrand equation modified by Wolfe A, et al. [42]. The equation is as below:

$$[DNA]/(\varepsilon_a - \varepsilon_f) = [DNA]/(\varepsilon_a - \varepsilon_f) + 1/K(\varepsilon_b - \varepsilon_f) \quad (1)$$

where, absorption coefficients, ε_a, ε_f, and ε_b correspond to A_{obs}/[compound], extinction coefficient for the compound and the extinction coefficient for the compound in the fully bound form, respectively. The binding constants for the different compounds (K_b) were determined by the slopes and the intercepts of the plots of [DNA] / (ε_a - ε_f) *vs.* [DNA].

Antioxidant Activity

The stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was used to assess the antioxidant activities of the plant extracts fractions using the standard modified method [43]. A solution of ascorbic acid (1.0 g/L) was prepared in methanol. The solution of DPPH (0.04%) was prepared in methanol, which was used as standard. The stock solutions of the plant extracts (400 mg) of the three extracts in methanol, ethyl acetate and acetone were taken and dissolved in 1.0 L methanol, separately and respectively. These were diluted to 300 200, 100 mg/L. To carry out the experiment, the absorbance and λ_{max} of pure DPPH were recorded. Later, 3.0 mL of DPPH was mixed with 2.0 mL of ascorbic acid and the absorbance and λ_{max} were recorded immediately. The same experiments were repeated with DPPH and the three plant extracts at different concentrations i.e. 400, 300 200, and 100 mg/L were used. All these solution mixtures were kept in dark for 30 min. and optical density was measured at 517 nm using UV/Vis spectrometer. The blank used was methanol (1.0 mL) having DPPH (0.04%, 1.0 mL). The blank solutions were used to correct values for radical decay. All the measurements were carried out for five times (n = 5). The concentration of the sample required to inhibit 50% of the DPPH free radical is called as IC_{50} value of the sample. This was calculated using the log dose inhibition curve. Low absorbance of the mixture of the sample solution and DPPH indicated high free radical activity of the reaction mixture [44].

RESULTS AND DISCUSSION

Preparation of Plant Extracts

During the extraction procedure, it was observed that the colors of the extracting solvents were different after the complete extraction. This observation clearly indicated that the various solvents have different constituents of the plant materials. It was due to the different polarities of the extracting solvents. Furthermore, the color of the extracting

solvents became dark on reducing the volumes of the extracting solvents by rotary evaporator apparatus under vacuum.

Anticancer Activities

The anticancer activities were measured with MTT assay and the results are presented in Figures 2 and 3 for H-1299 cancer cell lines and MCF-7 cancer cell lines, respectively. The anticancer activity of all the plant extracts against cancer cell lines increased with increase in the concentration (Figures 2 and 3). The anticancer activities of the plant extract were in the order of 200>150>100>50 µg/mL concentrations. The percentage of anticancer activities for lung cancer cell lines in terms of viability were in the range of 2.16 to 47.50% for all the extracts. The order of anticancer activities of the plant extracts was ETAC (P) >MeOH (P) > ACN (P) >MeOH (S) > Acetone (P) > Water-MeOH (P) > Water-MeOH (S) > Water (P). Among them, ethyl acetate extract was found more active with anticancer activities in the range of 2.16 to 32.68% (viability) for all the concentrations used. The IC_{50} and Hill's constant values for lung cancer cell lines were 74.48 to 902 µg/mL and 0.95 to 23.95 (Table 1). It is clear from this table that ethyl acetate extract was the best one with minimum value of IC_{50} and higher value of Hill's coefficient. These values were in good agreement of DNA binding studies results.

The percentage anticancer activities for breast cancer cell lines of all the plant extracts were in the range of 0.23 to 97.30 (viability). The order of anticancer activities was Acetone (P) >ETAC (P)>MeOH (P) > ACN (P)> Water-MeOH (P) > Water (P) >MeOH (S) >MeOH-Water (S). The best results were with acetone extract with anticancer activities in the range of 0.230 to 33.84% (viability) for all the concentrations. The IC_{50} and Hill's constant values for breast cancer cell lines were 51.59 to 240 µg/mL and 3.18 to 47.93 (Table 2). All these values are an indication of good activities of these plant extracts towards breast cancer cell lines. A comparison of the plant extracts activities on lung and breast cancer cell lines was carried out. It was observed that the plant extracts were more active against breast cancer in comparison to lung cancer. Of course, the plant extracts are working by interaction with DNA and both cell lines have almost similar DNA binding, but the anticancer activities are different. Therefore, it may be concluded that the plants extract not only interacted with DNA but also effecting some other phase of cell division. The best plant extracts were ethyl acetate and acetone in case of lung and breast cancer cell lines, respectively. It may be due to the fact that these solvents have different capacities for the extraction of the various chemical constituents. Furthermore, it may be concluded that the nature of the abnormal DNAs of both the cancer lines is different and, hence, the different effects of the plant extracts on the cancer cell line were observed.

Fractionation of Plant Extracts and their Anticancer Activities

Naturally, the plant extracts have many chemical constituents and it is essential to separate them to obtain the pure chemical compound. For this purpose, fractionation of ethyl acetate and acetone extracts was performed on Flash chromatography. Only three and two fractions were obtained in the case of ethyl acetate and methanol extracts. The mobile phase composition for each fraction is given in Table 3. The anticancer activities of these fractions were carried out and are given in Figures 4 and 5. The anticancer activities of the three fractions of ethyl acetate extracts were in the range of 50.74 to 68.32% (viability) (1st fraction), 65.69 to 89.48% (viability) (2nd fraction) and 71.96 to 93.58% (viability) (3rd fraction) for all the different concentrations. The IC_{50} and Hill coefficient values are given in Table 4. The range of IC_{50} and Hill's coefficient values were in 119.76 to 2310 µg/mL and 1.88 to 6.98, respectively. In the same way, the anticancer activities of the two fractions of acetone extracts were in the range of 55.74 to 72.32% (viability) (1st fraction) and 49.69 to 77.48% (viability) (2nd fraction) for all the different concentrations. The IC_{50} and Hill coefficient values are given in Table 4. The range of IC_{50} and Hill's coefficient values were in 1.72 to 9263 µg/mL and 0.27 to 1.36, respectively.

A perusal of these results indicated that the results are not better than the results of the combined extract. These findings clearly indicated that the overall anticancer activities are because of the combinations of the constituents present in the extracts. These results are supported by the earlier study of Aisha et al., according to whom phytochemicals in the combined form are more effective as compared to single phytochemicals when used to cure a disease.[45]

DNA Binding Studies

As usual DNA binding study is one of the important tools to find out the anti-cancer activity of any compound or extract. Therefore, the electronic absorption bands of the plant extracts (ethyl acetate and acetone) in the absence and presence of DNA are recorded and shown in Figure 6. The different values of λ_{max} (nm) of free plant extracts (λ_f), plant extracts bonded to DNA (λ_b), change in wavelengths after binding with DNA ($\Delta\lambda_{max}$), absorbance of free plant extracts (A_f), plant extracts bonded to DNA (A_b), change in absorbance after binding with DNA (ΔA), % hyperchromism (H%) and binding constants [K_b (M^{-1})] are given in Table 5. An evaluation of this Table indicates that the absorption spectra of free plant extracts ranged from 200 to 220 nm. After binding with DNA, the plant extracts absorptions were observed in the range of 200 to 208 nm. The comparison of λ_{max} values of free plant extracts (200 to 220 nm) and after binding with DNA (200 to 208) confirmed Hypsochromic or blue shifts. The changes in shifts were in the range of 3.0 to 20 nm, which are due to $\pi \rightarrow \pi^*$ transitions [46,47]. The

absorbance of free plant extracts was in the range of 0.11 to 0.49 and these were changed to 0.23 to 0.52 (Hyperchromic shifts) on the addition of different concentrations of DNA (30.0×10^{-4} - 1.0×10^{-5} M). The changes in the absorbance were in the range of 0.03 to 0.15. The % hyperchromisms were in the range of 6.12 to 90.90, which is an indication of DNA double helix structure damage [48,49]. These findings (results) are a strong indication of the plant extracts binding with DNA (DNA adducts formation) through electrostatic interactions with DNA in outside grooves [50,51].

It is important to mention here that these plant extracts formed an adduct with DNA; leading to deactivation of DNA activities. This observation is very important and useful to explain how the plant extracts will control cancer spreading. The values of DNA binding constants for ethyl acetate and acetone were 4.02×10^2 and 3.80×10^2; indicating good interactions with DNA. These results confirmed good binding characteristics of the plant extracts with DNA. The regression analysis was carried out using Origin software for DNA binding studies. The correlation coefficients (R^2) were in the range of 0.99874-0.99995. The values of regression coefficients were close to unity indicating the correctness of the experiments. Of course, the magnitudes of the DNA binding constants are quite high, which indicated that these extracts may be highly active against various cancers. Furthermore, it was also observed that the plant extract in ethyl acetate showed a higher value of DNA binding constant and, hence, better anticancer activity. The different values of DNA binding constants are due to the different polarities of the extraction solvents, which extracted various plant constituents at different degrees. Furthermore, high values of binding constants may be due to the presence of aromatic molecules having heteroatoms in the plant extracts constituents, as these heteroatoms have good tendency of interactions with DNA [52]. These results clearly indicated that ethyl acetate extract work through DNA binding on lung cancer line while in the case of breast cancer the anticancer activity is not only because of DNA binding but with some other mechanism.

Anti-oxidant Activities

The anti-oxidant activities of ethyl acetate and acetone plant extracts were carried out by standard method [53,54]. Both the tested extracts had exhibited scavenging effects on DPPH free radical. These results in terms of IC_{50} values are shown in Figure 7, which shows the scavenging activities of the selected extracts on DPPH radicals at various concentrations. The IC_{50} values calculated for EtAc and acetone extracts and ascorbic acid were 18.89 and 15.07 and 373.73 mg/L. It was noted that the scavenging activities of the EtAc and acetone extracts of *Cuscuta reflexa* Roxb. at all different concentrations were quite good. By comparing the antioxidant activities of ascorbic acid, it was observed that the different extracts have greater antioxidant activities than ascorbic acid. The results calculations depicted clearly that EtAc and acetone extracts had the strongest anti DPPH radical activities. These results are due the presence of various antioxidant molecules in the plant extracts. These compounds show the prominent reducing properties against DPPH radical. The powerful antioxidant role of the extracts may be credited to their strong hydrogen donating capability, metal chelating property and scavenging of free radicals like hydrogen peroxide and superoxide (Table 6) [55].

CONCLUSION

Finally, it was concluded that all the extracting solvents were capable to extract the plant material constituents at different extents. The anticancer activities of plant extracts were checked. The percentage of anticancer activities for lung cancer cell lines was in the range of 2.16 to 47.50 for all the extracts. Among them, ethyl acetate extract was more effective with anticancer activities in the range of 2.16 to 32.68% for all the concentrations used. The IC_{50} and Hill's constant values for lung cancer cell lines were 74.48 to 902 µg/mL and 0.95 to 23.95. The results values were in good agreement of DNA binding studies results. On the other hand, the percentage of anticancer activities for breast cancer cell lines was in the range of 0.23 to 97.30. Among all plant extracts, acetone extract was more effective with anticancer activities in the range of 0.230 to 33.84 % for all the concentrations. The IC_{50} and Hill's constant values for breast cancer cell lines were 51.59 to 240 µg/mL and 3.18 to 47.93. It was observed that the plant extracts showed greater anticancer activities in breast cancer cell lines as well as lung cancer cell lines as compared to fractions obtained from extracts using flash chromatography. Finally, it was concluded that the plant extract may be useful to control the breast and lung cancer both.

The plant extracts interacted with DNA resulting in good binding constants. It was also observed that all the plant extracts damaged DNA structure – an indication of inhibition of cancerous cell growth. This activity of plant extracts may be responsible to check the growth of cancerous cells. The order of binding constants was III> IV> II > I > V> VI > VII > VIII. These results confirmed good binding characteristics of the plant extracts with DNA. Of course, DNA binding constants of the plant extracts of both stems and seeds are quite good. Moreover, it is also clear from these results that the extracts of stem interacted more strongly with DNA. Further, these plant extracts were screened for anticancer activities with lung cancer cell lines (H-1299) and breast cancer (MCF-7) cell lines. The antioxidant activities of ethyl acetate and acetone extracts showing higher anticancer potential were also checked. Each extract showed different antioxidant activity with respect to ascorbic acid. Finally, it was concluded that the ethyl acetate and acetone extract of this plant may be

used to prepare the Unani medicine for treating lung and breast cancers without any toxicity.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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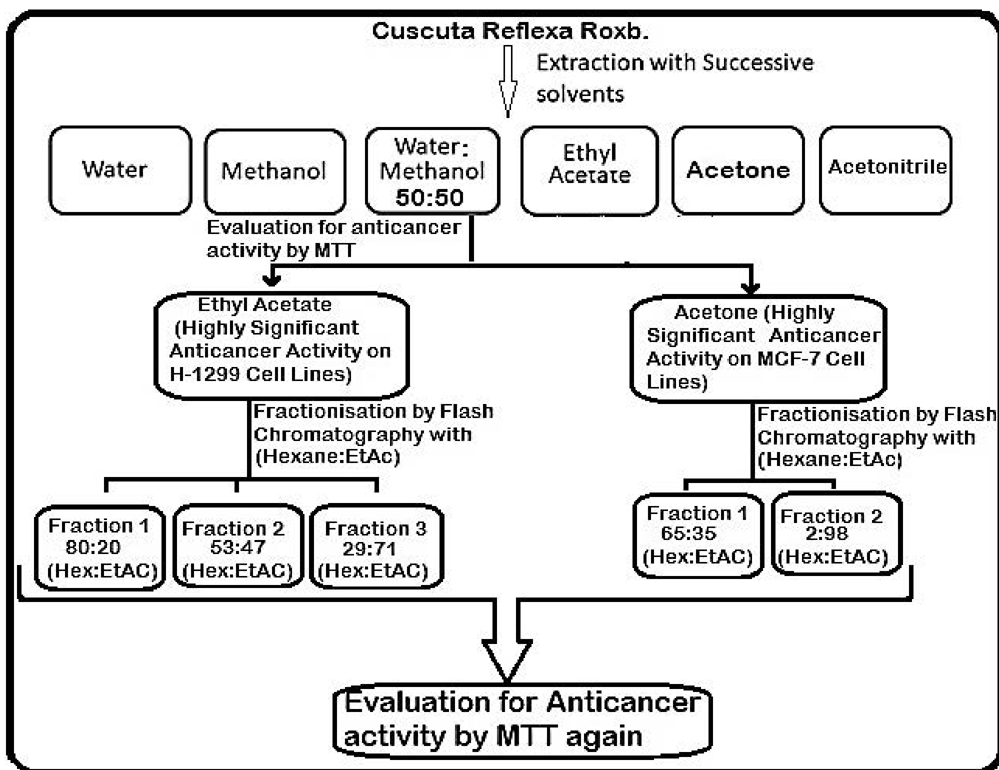

Figure 1. The experimental protocol.

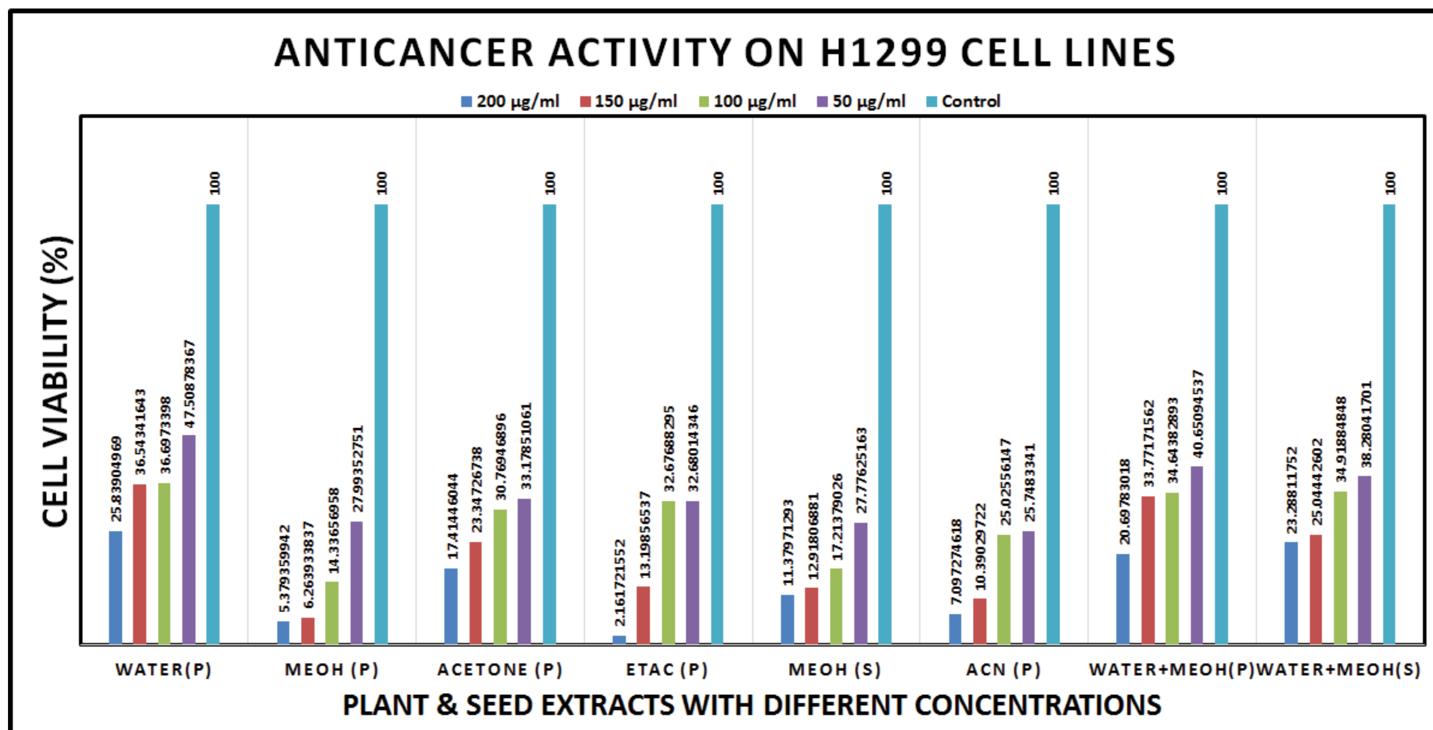

Figure 2. Anticancer activity of plant extract on H-1299 cancer cell lines [(P)=Stem and (S)=Seed].

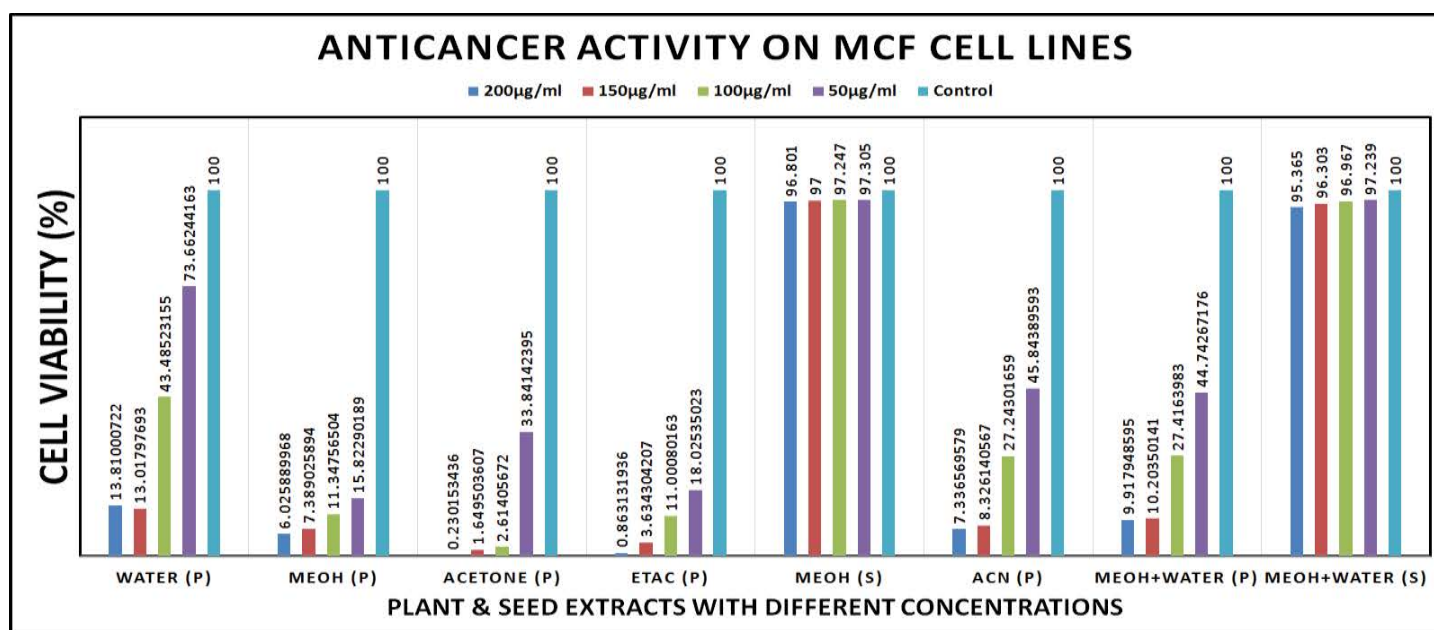

Figure 3. Anticancer activity of plant extract on MCF-7 cancer cell lines [(P)=Stem and (S)=Seed].

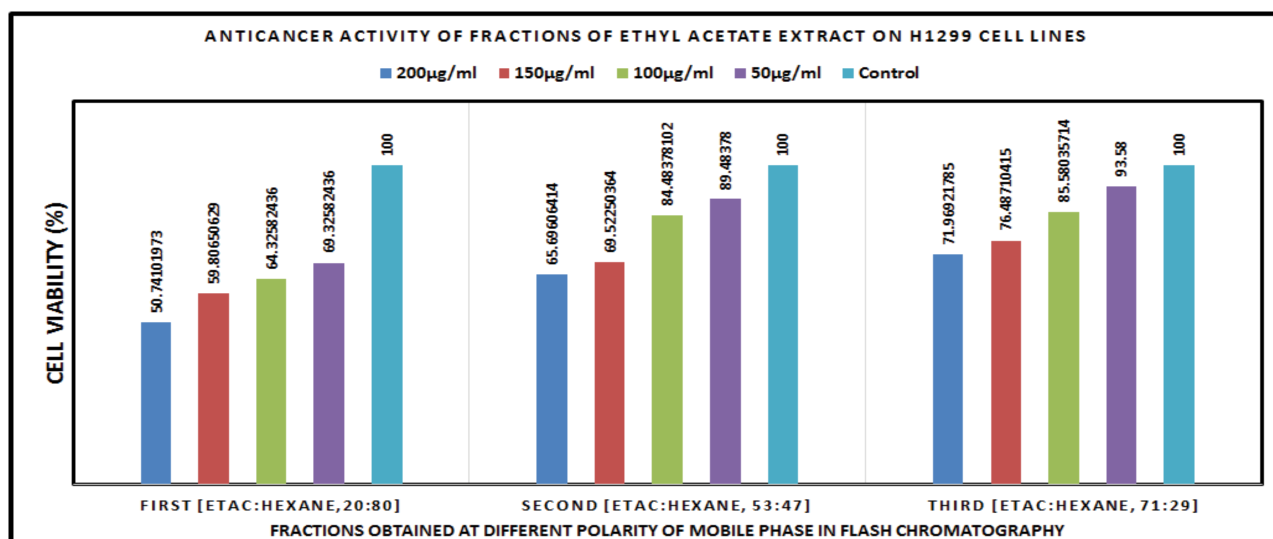

Figure 4. Anticancer activity ethyl acetate plant extract fractions on H-1299 cancer cell lines.

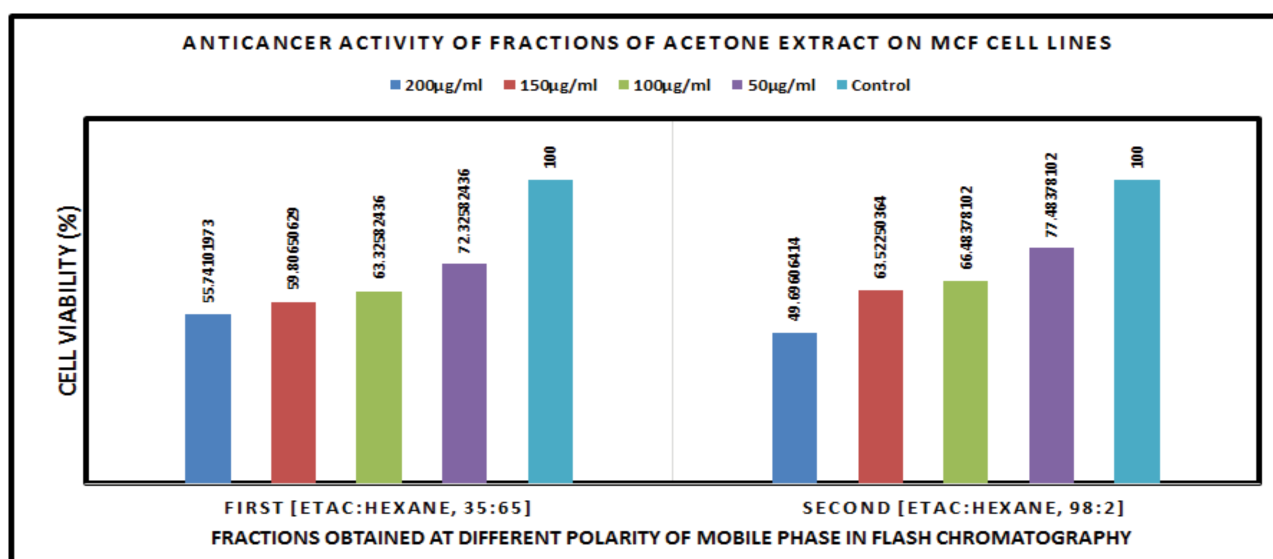

Figure 5. Anticancer activity acetone plant extract fractions on MCF Cancer cell lines.

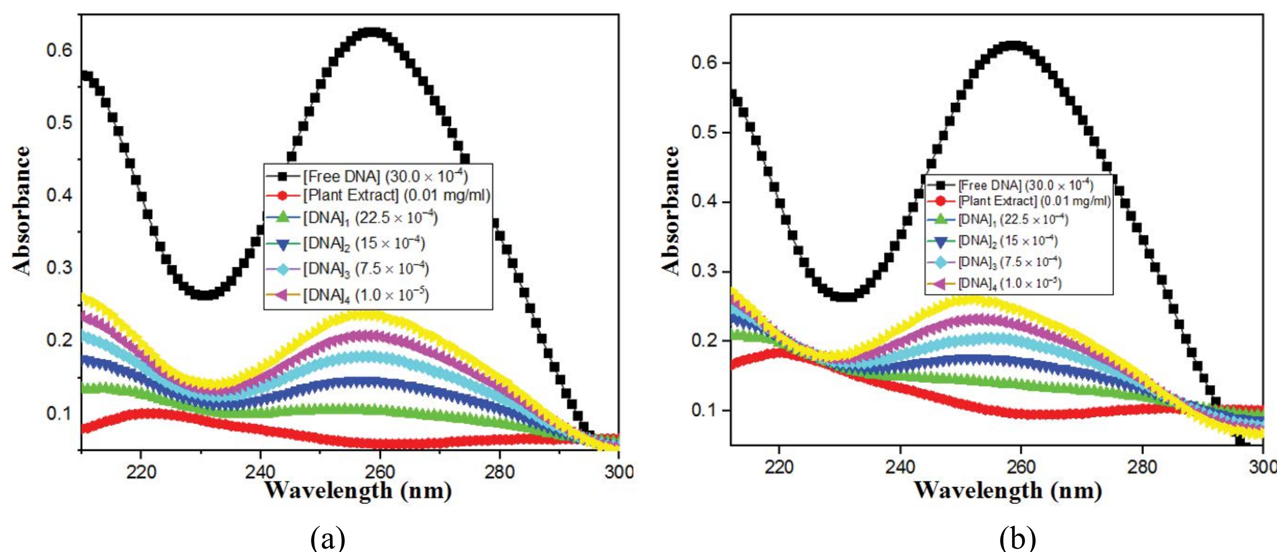

Figure 6. DNA binding spectra with plant extract in (a) ethyl acetate and (b) acetone.

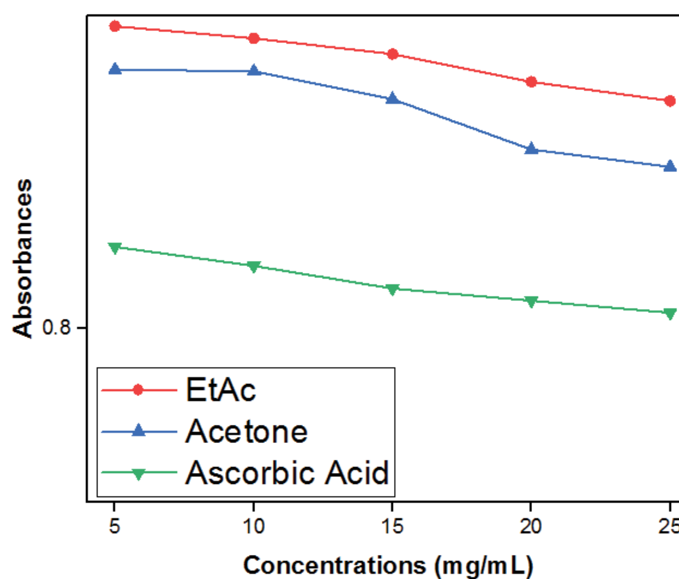

Figure 7. Concentrations of scavenging of DPPH free radical by ascorbic acid, MeOH, EtAc and Acetone. The reaction mixture (4.0 mL) in methanol contained DPPH in the presence of MeOH, EtAc, Acetone and Ascorbic acid. The absorbance was measured at 517 nm.

Table 1. IC_{50} and Hill coefficient data on H-1299 cell lines for all the plant extracts.

Plant Extracts	IC_{50} (μg/mL)	Hill's Coefficient
Stem Acetone (I)	150	4.7
Stem ACN (II)	131	11.62
Stem ETAc (III)	74.48	23.95
Stem MeOH (IV)	93.50	6.43
Stem H_2O + MeOH (V)	571	3.37
Stem Water (VI)	902	0.95
Seed MeOH (VII)	146	2.77
Seed Water+ MeOH (VIII)	117	7.76

Table 2. IC_{50} and Hill coefficient data on MCF-7 cell lines for all the plant extracts.

Plant Extracts	IC_{50} (μg/mL)	Hill's Coefficient
Stem Acetone (I)	51.59	5.13
Stem ACN (II)	100.66	8.93
Stem ETAC (III)	110.66	4
Stem MeOH (IV)	104.99	4
Stem H_2O+MeOH (V)	100.08	11
Stem Water (VI)	99.99	47.99
Seed MeOH (VII)	149	5.52
Seed Water+MeOH (VIII)	240	3.18

Table 3. Fractionation of plant extracts and anticancer activity of each fraction.

Plant Extracts	Fractions	Hexane-EtAc
EtAc	Fraction 1	80:20, v/v
	Fraction 2	53:47, v/v
	Fraction 3	29:71, v/v
Acetone	Fraction 1	65:35, v/v
	Fraction 2	2:98, v/v

Table 4. IC_{50} value and Hill's coefficients values of the different fractions of the ethyl acetate and acetone plant extracts.

Extracts used	Fractions	IC_{50} (μg/mL)	Hill's Coefficient
Ethyl acetate	First Fraction	2310	1.88
	Second Fraction	121	6.98
	Third Fraction	119.76	3.5
Acetone	First Fraction	1.72	0.27
	Second Fraction	9263	1.36

Table 5. DNA binding studies of the plant extracts.

Plant Extracts	Λ_f (nm)	Λ_b (nm)	$\Delta\lambda_{max}$ (nm)	A_f	A_b	ΔA	(H%)	K_b (M^{-1})
ETAc (III)	220	200	20	0.19	0.25	0.06	31.57	4.02×10^2
Acetone (I)	220	208	12	0.11	0.23	0.12	90.9	3.80×10^2

% Hyperchromism (H%)=[Change in absorbance/Af] × 100, where Af and Ab represent The absorbance of free and bound plant extracts; λf=λmax of free extracts, λb= λmax of extracts bound to DNA and Kb=Binding constants.

Table 6. Anti-oxidant IC_{50} value of ascorbic acid, EtAc and acetone plant extracts with DPPH free radical.

Plant Extracts	IC_{50}(mg/mL)	Hill's Coefficient
EtAc Extract	18.89	7.55
Acetone Extract	15.07	2.26
Ascorbic Acid	373.73	1.87