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In Vitro Antioxidant and Anticancer Activity of *Andrographis serpyllifolia* (Rottler Ex Vahl) Wight on Human Cancer Cell Lines.

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Abstract: *Andrographis serpyllifolia* (Rottler ex Vahl) Wight (*Acanthaceae*) is the plant which found in South India in the state of Karnataka, Tamil Nadu and Andhra Pradesh shows various biological activities. Whole plant subjected to extraction and followed by fraction. Phytochemical investigation was carried out using different biochemical tests. Total phenolics in extract and fractions were determined quantitatively by Folin-Ciocalteau reagent method. The antioxidant activity was evaluated by Diphenyl Picryl Hydrazyl (DPPH) radical scavenging method, nitric oxide radical inhibition method, reducing power method. *In vitro* anticancer activity on MCF-7, A549, and HEK 293 cancer cell lines was carried out by (3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) MTT assay. The Phytochemical investigation confirmed the presence of alkaloids, glycosides, phenolic compounds, flavonoids, tannins, sterols, saponins, fixed oils and fats, protein and amino acids, Gum and mucilage, and carbohydrates. Ethanol extract followed by ethyl acetate fraction were shown highest antioxidant activity. The ethanol extract significantly reduced the percent viability of MCF-7, A549 and HEK 293 cancer cell lines with IC_{50} value 410 ± 11.547 µg/ml for MCF-7, 428.34 ± 10.137 µg/ml for A549 and 426.67 ± 8.819 µg/ml for HEK 293.

Key words: *A. serpyllifolia*, phytochemical, antioxidant, anticancer, cancer cell lines.

1. Introduction

Cancer is a major public serious problem in both developed and developing countries. Generally cancer cells attack and destroy normal cells. It is an abnormal growth of cells in body that can lead to death. Despite of spending billions of dollars on cancer research and development of world class improved diagnostic

techniques since few decades, we do not understand cancer in detail and disease still impacts millions of patients all over the world. [1]

A free radical is unstable possess an unpaired electron. It can damage and destroy a protein, enzyme or a complete cell. In worst condition, free radicals can multiply through a chain reaction with the release of thousands of oxidants and cells damaged badly that DNA codes can be changed and immunity can be altered. Diseases like cancer can be resulted on repeatedly contact with a free radical. [2] Some of the examples of dangerous free radical producing substances includes: cigarette smoke, herbicides, high fats, pesticides, smog car exhaust, some prescription drugs, x-rays , ultraviolet light , gamma radiation , rancid foods, certain fats, stress, poor diets etc. Exercising, also initiate the release of free radicals within our cellular systems. Aerobic exercising produces damaging oxidants. Many of these free radicals are not completely neutralized by internal safety mechanisms and an over load can occur. Supplementing the diet with effective antioxidant compounds can reduce the free radicals. The role of certain bioflavonoid compounds were found exceptional free radical scavengers. [3]

Although the currently available therapies for cancer include chemotherapy, radiotherapy and surgical operation have saved lives of numerous cancer patients, the severe side effects make them only partially effective to cure and control cancers, and therefore the demand for discovery and development of more effective cancer therapies and biologically active entities from different sources is increasing. On Comparison with synthetic chemotherapeutic drugs, plant-derived natural molecules are relatively less toxic, possess high target specificity.[4] Thus, the alternate solution for the harmful effects of synthetic drugs is the use of complementary alternative medicines as very few studies have been reported on the use of herbal medicine in treatment of cancer.[5]

Andrographis serpyllifolia (Rottler ex Vahl) Wight (*Acanthaceae*) occurs in hill top regions in South India in the state of Karnataka, Tamil Nadu and Andhra Pradesh. [6, 7]

Based on the literature survey, the plant shows antidiabetic [8], anti-inflammatory [9, 10], antibacterial [11], antimicrobial [12], antioxidant [2], antityphoid [13] activities. However, there are very few available studies on anticancer and antioxidant properties of this plant. Thus, we have made an attempt to use this herbal plant to check the efficacy against human cancer cell lines. The present study was aimed to evaluate preliminary phytochemical, *in vitro* antioxidant properties, and *in vitro* anticancer potential activities against MCF-7, A549 and HEK293 human cancer cell lines.

2. Methods

2.1. Collection and authentication of plant part

Whole plant of *A. serpyllifolia* was collected in the month of August, from Andhra Pradesh (India). Herbarium specimens were prepared. Identification and authentication were done by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri. Venkateswara University, Tirupati, A. P. India. A Voucher specimen number of the plant 1189 has been deposited for future reference. The plant material was shade dried and

coarsely powdered by using mechanical grinder. The powder was passed through sieve no. 40 and stored in airtight container for the extraction.

2.2. Preparation of plant materials

The air dried whole plant (1000 g) was extracted with ethanol (70 % v/v) and concentrated in rotary evaporator under reduced pressure to get ethanol extract (192 g) and designated as ethanol extract (EE). Fractionation of the ethanol extract was done by solvent-solvent partition. [14] Five grams of the ethanol extract was dissolved in 200 ml hot ethanol. Slight precipitation obtained was discarded as ethanol insoluble matter. The ethanol soluble fraction was filtered and collected. It was concentrated to about 50 ml volume and chloroform was added to it. Fraction carried out in separation funnel. Then chloroform layer was separated and this procedure repeated 7-8 times. This chloroform fraction was concentrated further to dryness and designated as chloroform fraction (CF). Then process was repeated for same ethanol extract in the separation funnel for fractionation with ethyl acetate and designated as ethyl acetate fraction (EA).

2.3. Phytochemical analysis

The EE, CF and EA were subjected to qualitatively tests for different phytochemical constituents namely alkaloids, flavonoids, glycosides, phenolic compounds, saponins, sterols, tannins, fixed oils and fats, protein and amino acids, gum and mucilage and carbohydrates by following the standard procedure. [15]

2.4. Estimation of total phenolic content

Total soluble phenolics in the extracts were determined with Folin-Ciocalteau reagent according to the method of Slinkard and Singleton [16] using gallic acid as a standard phenolic compound. The extract (10 mg/10 ml) was dissolved in water, from that it was diluted to get concentration of 100 µg/ml. 0.1 ml from above solution was transferred in 10 ml volumetric flask. 1.0 ml of Folin-Ciocalteau reagent was added and the content of the flask mixed thoroughly. 3 min later 3.0 ml of 2% sodium carbonate was added and volume was made with distilled water, the mixture was allowed to stand for 2 hours with intermittent shaking. The absorbance of the blue color that developed was read at 760 nm spectrophotometrically using distilled water as a blank. The concentration of total phenols was expressed as mg/g of dry extract. [17] The concentration of total phenolic compounds in the extract was determined as mg of gallic acid equivalent (GAE) using an equation obtained from the standard gallic acid graph. Results were expressed percentage w/w and calculated using following formula, Total phenolic content (% w/w) = $GAE \times V \times D \times 10^{-6} \times 100/W$, GAE - Gallic acid equivalent (µg/ml), V - Total volume of sample (ml), D - Dilution factor, W - Sample weight (g). [18]

2.5. Determination of antioxidant activity by using in-vitro methods

2.5.1. Diphenyl Picryl Hydrazyl (DPPH) radical scavenging method [19, 20, 21, 22]

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used are 1000 to 1.95 µg/ml. The plates were incubated at 37 ºC for 20 minutes and the absorbance of each solution was measured at 518 nm against the corresponding test and standard blanks and the remaining DPPH was calculated. IC_{50} (Inhibitory Concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals.

Formula

$$IC_{50} \text{ value} = \{\text{Control} - \text{Sample}/ \text{Control}\} \times 100$$

2.5.2. Nitric oxide radical inhibition activity [23, 24]

The reaction mixture (6 ml) containing sodium nitroprusside (4 ml), phosphate buffer saline (PBS, 1 ml) and 1 ml of extract in DMSO were incubated at 25ºC for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent was added, mixed well and allowed to stand for 5 minutes for completion of diazotization, then 1 ml of Naphthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of these solutions was measured at 546 nm against corresponding blank solution. IC_{50} value obtained is the concentration of the sample required to inhibit 50% nitric oxide radical. Ascorbic acid was used as positive control.

$$\text{NO scavenged (\%)} = \frac{\text{Acont} - \text{Atest}}{\text{Acont}} \times 100$$

Where A_{cont} is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts.

2.5.3. Reducing power method [25]

The reducing power of the extracts was determined according to the method of Oyaizu. Various concentration of the extracts (100-1000 µg/ml) in 1.0 ml of deionised water were mixed with phosphate buffer (2.5 ml, 0.2 M pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Reducing power is given in ascorbic acid equivalent ($ASEml^{-1}$)

that shown the amount of ascorbic acid expressed in mM those reducing power is the same than that of 1 ml sample.

2.6. Culturing of cell lines

The cell lines MCF-7, A549, and HEK 293 were procured from the National Centre for Cell Science, Pune, India. The human breast cancer cell line MCF-7 and human lung adenocarcinoma epithelial cell line A549 were cultured in DMEM with low glucose, and Human Embryonic Kidney (HEK) 293 cells were cultured in Serum-free HEK 293 growth medium supplemented with 10 % fetal bovine serum and incubated in humidified atmosphere of 5 % CO_2 and 37 °C. The culture medium was changed every two days.

2.7. MTT cell viability assays

The cells were seeded at a density of approximately 5×10^3cells/well in a 96-well flat-bottom micro plate and maintained at 37ºC in 95% humidity and 5% CO_2 for overnight. Different concentration (500, 250, 125, 62.5, 31.25, 15.625 µg/ml) of EE, CF and EA were treated. The cells were incubated for another 48 hours. The cells in well were washed twice with phosphate buffer solution, and 20 µl of the MTT staining solution (5 mg/ml in phosphate buffer solution) was added to each well and plate was incubated at 37^0C. After 4h, 100 µl of di-methyl sulfoxide (DMSO) was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader.[26] The effect induced was also compared with the standard drugs used, namely, paclitaxel. The results were analyzed in triplicate and the percentage was calculated.

Formula:

Surviving cells (%)
= Mean OD of test compound /Mean OD of Negative control x 100

2.8. Statistical analysis

The results were expressed as mean ± standard deviation. Descriptive statistics was used to analyze the mean, standard deviation, variation, and level of statistical significance between groups. When $p < 0.05$ and $p < 0.01$, it was considered statistically significant for analysis of percent of surviving cells.

3. Results

3.1. Total yield of extract and fractions

The total yield of ethanolic extract, chloroform and ethyl acetate fractions of *A. serpyllifolia were* 19.2 % w/w, 9.8 % w/w and 9.4 % w/w respectively.

3.2. Phytochemical analysis

Preliminary screening of *A. serpyllifolia* extract and fractions showed the presence of diversity of phytochemical constituents. The ethanolic extract of *A. serpyllifolia* reveals the presence of alkaloids, tannins, gums and mucilage, carbohydrates and saponins. Whereas sterols, proteins and amino acids, fixed oil and fats are found in both ethanol extract and chloroform fraction. Presence of glycosides found in ethyl acetate fraction and ethanol extract. Phenolic compounds and flavonoids are showed their presence in ethyl acetate fraction; chloroform fraction as well as ethanol extract. (Table 1)

Table 1 – Preliminary phytochemical screening of *Andrographis serpyllifolia* extract and fractions.

Sr. No.	Phytoconstituents	Ethanol extract (EE)	Chloroform fraction (CF)	Ethyl acetate fraction (EA)
1	Alkaloids	+	-	-
2	Sterols	+	+	-
3	Glycosides	+	-	+
4	Fixed oils and fats	+	+	-
5	Phenolic compounds	+	+	+
6	Protein and amino acids	+	+	-
7	Tannins	+	-	-
8	Gum and mucilage	+	-	-
9	Flavonoids	+	+	+
10	Carbohydrates	+	-	-
11	Saponins	+	-	-

+, Presence; –, Absent

3.3. Total phenol content

In this present study, the phenol content in extract and fractions was determined by calorimetric method by using Folin-Ciocalteus reagent at 760 nm in percent of w/w of extract. In the chloroform fraction polyphenol content was found to be 0.04 ± 0.00029 % w/w, in ethyl acetate fraction 0.07 ± 0.000691 % w/w and in alcohol extract 0.09 ± 0.000742 % w/w.

3.4. DPPH assay

In the present study, the different concentrations of ethanol extract, chloroform fraction and ethyl acetate fraction of plant of *A. serpyllifolia* were subjected to DPPH free radical scavenging assay. The antioxidant capacity of the extract and fractions were compared with ascorbic acid as the standard antioxidant. Ethanol extract exhibited higher antioxidant activity with IC_{50} value 48 µg/ml than the chloroform and ethyl acetate fractions with IC_{50} value 380 µg/ml and 122 µg/ml respectively. (Table 2)

Table 2: Determination of percentage inhibition of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *A. serpyllifolia*.

Concentration µg/ml	Ethanol extract	Chloroform fraction	Ethyl acetate fractions
0	0	0	0
50	52.83 ± 0.69	43.39 ± 0.83	39.62 ± 0.79
100	58.49 ± 0.82	47.17 ± 0.79	45.28 ± 0.84
200	64.15 ± 0.84	49.06 ± 0.81	67.79 ± 0.86
400	81.13 ± 0.79	50.94 ± 0.69	69.81 ± 0.78
600	83.01± 0.89	62.26 ± 0.75	75.47 ± 0.88
800	84.9 ± 0.96	66.04 ± 0.72	79.24 ± 0.81
1000	86.2 ± 0.98	72.01 ± 0.74	84.2 ± 0.94
IC_{50} value	48 µg/ml	380 µg/ml	122 µg/ml

The values presented are mean ± standard deviation, $n = 3$.

3.5. Nitric oxide radical inhibition activity of *A. serpyllifolia*

In the present study, the different concentrations of ethanol extract, chloroform fraction and ethyl acetate fraction of plant of *A. serpyllifolia* were subjected to nitric oxide radical inhibition activity. The antioxidant capacity of the extract and fractions were compared with ascorbic acid as the standard antioxidant. Ethanol extract exhibited higher antioxidant activity with IC_{50} value 83 µg/ml than the chloroform and ethyl acetate fractions with IC_{50} value 108 µg/ml and 136 µg/ml. (Table 3)

Table 3: Determination of percentage inhibition of nitric oxide radical inhibition activity of *A. serpyllifolia*

Concentration µg/ml	Ethanol Extract	Chloroform fraction	Ethyl acetate fraction
0	0	0	0
50	42.83 ± 0.79	33.39 ± 0.64	38.52 ± 0.69
100	53.49 ± 0.86	49.07 ± 0.85	46.18 ± 0.78
200	66.55 ± 0.94	59.16 ± 0.89	56.59 ± 0.82
400	71.43 ± 0.88	62.94 ± 0.93	62.01 ± 0.89
600	73.41 ± 0.91	68.62 ± 0.96	77.47 ± 0.92

800	80.19 ± 0.94	70.14 ± 0.91	80.34 ± 0.93
1000	86.22 ± 0.98	73.51 ± 0.95	84.82 ± 0.96
IC 50 value	**83 µg/ml**	**108 µg/ml**	**136 µg/ml**

The values presented are mean ± standard deviation, *n* = 3.

3.6. Reducing power of *A. serpyllifolia*

In the present study, the presence of antioxidants in the sample would result in the reduction of ferri cyanide $Fe3^{+}$ to ferro cyanide $Fe2^{+}$ by donating an electron. Ethanolic extracts, chloroform and ethyl acetate fractions were subjected to FRAP assay along with standard ascorbic acid. In the results obtained, ethanol extract showed higher activity than ethyl acetate fraction fractions followed by chloroform fraction which was comparable to standard ascorbic acid (Table. 4).

Table 4: Determination of reducing power of extract and fractions *A. serpyllifolia* and ascorbic acid

Sr. No	Concentration µg/ml	Ethanol extracts	Chloroform fraction	Ethyl acetate fraction	Ascorbic acid
1	100	0.054	0.015	0.045	0.204
2	200	0.428	0.042	0.072	0.318
3	400	0.535	0.083	0.183	0.485
4	600	0.781	0.128	0.428	0.681
5	800	0.823	0.423	0.523	0.703
6	1000	0.924	0.524	0.724	0.884

The values presented are mean ± standard deviation, *n* = 3.

3.7. Effect of *A. serpyllifolia* ethanol extract, chloroform fraction and ethyl acetate fraction on MCF-7, A549, and HEK 293 cancer cells lines

The result of MTT assays revealed that the ethanol extract and ethyl acetate fraction of *A. serpyllifolia* decreased the percent viability of all the cells but to different extent. Cell viability was determined in the presence of increasing concentrations of plant extract and fraction of *A. Serpyllifolia.* Ethanol extract and ethyl acetate fraction was found to induce more cytotoxicity towards cancer cell lines MCF-7, A549 and HEK 293. (Table 5, 6, 7). The IC50 values of ethanol extract, chloroform fraction and ethyl acetate fraction on MCF-7, A549, and HEK 293 cancer cells lines are represented in (Table 8).

Table 5: Cell Viability (in %) of MCF 7 cell lines for ethanol extract, chloroform fraction and ethyl acetate fraction

Conc. µg/ml	Ethanol Extract	Chloroform Fraction	Ethyl acetate Fraction
500	49.45 ± 0.505	98.16 ± 0.139	61.53 ± 0.283
250	57.99 ± 0.157	99.11 ± 0.118	74.39 ±0.206
125	72.36 ± 0.328	99.10 ± 0.122	81.55 ±0.246
62.5	79.57 ± 0.307	99.82 ± 0.163	91.61 ±0.206
31.25	87.7 ± 0.325	100.00 ± 0.000	96.87 ±0.101
15.625	96.61 ± 0.242	100.00 ± 0.000	98.67 ±0.220

The values presented are mean ± standard deviation, *n* = 3.

Table 6: Cell Viability (in %) of A549 cell lines for ethanol extract, chloroform fraction and ethyl acetate fraction

Conc. µg/ml	Ethanol Extract	Chloroform Fraction	Ethyl acetate Fraction
500	48.54 ± 0.303	98.53±0.289	62.87±0.287
250	57.89 ± 0.107	99.39±0.376	75.02±0.033
125	71.93 ± 0.228	99.61±0.161	81.63±0.331
62.5	80.17 ± 0.224	99.94±0.045	92.02±0.078
31.25	88.10 ± 0.106	100.00±0.00	96.87±0.248
15.625	97.06 ± 0.070	99.96±0.036	99.03±0.032

The values presented are mean ± standard deviation, *n* = 3.

Table 7: Cell Viability (in %) of HEK 293 cell lines for ethanol extract, chloroform fraction and ethyl acetate fraction

Conc. µg/ml	Ethanol Extract	Chloroform Fraction	Ethyl acetate Fraction
500	47.97 ± 0.233	98.00 ± 0.199	63.55±0.538
250	59.61 ± 0.438	98.63 ± 0.184	76.77±0.168
125	72.10 ± 0.131	99.77 ± 0.165	80.73±0.224
62.5	83.72 ± 0.146	99.95 ± 0.046	93.47±0.250
31.25	89.78 ± 0.399	100.00 ± 0.000	97.47±0.291
15.625	97.94 ± 0.046	100.00 ± 0.000	98.93±0.171

The values presented are mean ± standard deviation, *n* = 3.

Table 8: IC_{50} values of ethanol extract, chloroform fraction and ethyl acetate fraction in µg/ ml

Extract/ Fractions	MCF-7	A549	HEK293
Ethanol extract	410 ± 11.547	428.34 ± 10.137	426.67 ± 8.819
Chloroform Fraction	1123.33 ± 14.529	1156.67 ± 23.334	1200.00 ± 34.641
Ethyl acetate Fraction	625 ± 14.433	643.34 ± 12.018	640.00 ± 25.166
Paclitaxel	12.33 ± 0.334	11.34 ± 0.881	12.34 ± 0.881

The values presented are mean ± standard deviation, *n* = 3.

4. Discussion

The current scenario revealed the use of herbal medicines in cancer treatment and received increasing attention due to their varied phytoconstituents with multiple biological activities. [27] The plant collected from Andhra Pradesh was identified according to their taxonomical characters as *A. serpyllifolia* and analyzed for the presence of phytochemicals.

Preliminary phytochemical analysis showed the presences of secondary metabolites in the extract and fractions of the plant. These secondary metabolites are reported to have many biological and therapeutic properties.[28] Phytoconstituents were determined qualitatively by different biochemical tests. Flavonoids and phenolic compounds were detected in ethanol extract, chloroform and ethyl acetate fractions whereas alkaloids, tannins, and saponins were present in ethanol extract. The presence of glycosides was shown in ethyl acetate fraction and ethanol extract. Sterols, fixed oil and fats, proteins and amino acids were detected in chloroform fraction and ethanol extract (Table 1). Among the various phytoconstituents, flavonoids and

phenolic compounds have applications in different areas such as pharmaceutical, health, food, and cosmetic industries. These compounds are widespread in the plant kingdom as part of our daily diet and are attractive as natural antioxidants. [29]

Phytochemicals have long been recognized to possess many properties including antioxidant, antiallergic, anti inflammatory, antiviral, anti-proliferative and anticarcinogenic. [30] In this study, the phenolic content was studied in *A. serpyllifolia* wherein the ethanol extract exhibited the highest total phenolic content (0.09 ± 0.000742 %) followed by the ethyl acetate fraction (0.07 ± 0.000691 %). Active oxygen species and other free radicals have long been known to be mutagenic. These agents have more recently emerged as mediators of the other phenotypic changes that lead from mutation to neoplasm. Therefore, free radicals may contribute widely to cancer development in humans. More interestingly free oxygen radicals are increasingly discussed as important factors involved in the phenomenon of biological aging. [31] Many secondary metabolites like phenols, polyphenols, and flavonoids serve as sources of antioxidants and perform scavenging activity. [32]

In the present work, three methods DPPH radical scavenging, nitric oxide radical scavenging and reducing power were used to evaluate the total antioxidant capacity of ethanol extract, chloroform and ethyl acetate fractions. DPPH assay based on the measurement of the scavenging ability of antioxidants towards the stable radical of DPPH. The free DPPH radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors; this ability is evaluated by more frequently used technique in the decolouration assay, which evaluates the absorbance decrease at 518-528 nm produced by the addition of the antioxidant to a DPPH solution in ethanol or methanol.[18,19,20,21] In the present study, the antioxidant activity of *A. serpyllifolia* was evaluated using ethanol extract, chloroform, and ethyl acetate fractions of the plant and was compared with standard ascorbic acid. The experimental data revealed that the extract and fractions are likely to have the properties of scavenging free radicals with ethanol extract showing higher antioxidant capacity followed by ethyl acetate fraction. (Table 2)

Sodium nitroprusside (SNP) in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be estimated by the use of Griess Illosvoy reaction. In the present investigation, Griess Illosvoy reagent is modified by using naphthyl ethylene diamine dihydrochloride (NEDD) (0.1 % W/V) instead of 1-naphthylamine (5%). Nitric oxide interacts with oxygen to produce stable products, leading to the production of nitrites. [22, 23] (Table 3)

The ferric ion-reducing power assay of the extract may show a significant indication of its potential antioxidant activity. The presence of antioxidants, which have been shown an antioxidant action by breaking the free radical chain by donating a hydrogen molecule. [33] The antioxidants present in the extract and fractions would result in the reduction of ferri cyanide Fe^{3+} to ferro cyanide Fe^{2+} by donating an electron which was measured spectrophotometrically at 700 nm. In this assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of plant extract. The reducing

power increased with the increase in the extract concentrations. Hence, this study presumed that the ethanol extract of *A. serpyllifolia* may have a high amount of antioxidant properties. (Table 4)

The anticancer activity of plant extracts is evaluated by different methods. The MTT assay is used in screening the extract and fractions to assess the possible cytotoxic properties of the same. MTT assay is based on the principle of reduction of MTT by mitochondrial dehydrogenase to dark purple coloured formazan product. It is frequently used as an *in vitro* method to measure cytotoxic effects of variety of toxic substances and plant extracts against cancer cell lines. [34] *In vitro* test using MCF-7, A549 and HEK 293 cancer cell lines was performed to screen potential cytotoxicity. The ethanol extract, chloroform and ethyl acetate fractions of *A. serpyllifolia* showed *in vitro* cell viability effects on the MCF-7, A549 and HEK 293 cancer cell lines.

This effect was concentration dependent. The percent viability of cells was reduced in a significant, inversely in dose response manner due to treatment extract and fractions. The extract and fractions with concentration of 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.625 µg/ml were evaluated in triplicate. Among these, 500µg/ml of ethanol extract and ethyl acetate fraction was most effective in reducing the percent viability of MCF-7, A549 and HEK 293 cancer cell lines while the response of chloroform fraction was weaker. However, the standard paclitaxel drug showing significant reduction in percent viability of the cancer cell lines. The results showed that ethanol extract significantly reduced the percent viability of MCF-7, A549 and HEK 293 cancer cell lines and was the most potent extract with IC_{50} value 410 ± 11.547 µg/ml for MCF-7, 428.34 ± 10.137 µg/ml for A549 and 426.67 ± 8.819 µg/ml for HEK 293 followed by ethyl acetate fraction (Table 8). Phenolics and andrographolide in *A. serpyllifolia* may be implicated in anticancer properties as observed in traditional therapies. [35]

5. Conclusion

In conclusion, it was observed that the plant *A. serpyllifolia* contains a variety of secondary metabolites that show strong antioxidant property based on the experiments performed which add scientific evidence to conduct further studies, investigate the lead biomolecules present in the plant, evaluate its anticancer potential on *in vivo* animal models and put forward an attempt to save human around the world from cancer.

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