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Research Article

Comparative Anticancer Activity of *Pelargonium graveolens* Extracts: Soxhlet vs.Maceration methods against HeLa cells

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ABSTRACT

Pelargonium graveolens L'Her. (Geranium) is a well known medicinal plant in South Africa that has been used for a long time as a traditional medicine. *Pelargonium graveolens* leaves yield aromatic volatile and nonvolatile essential oils which have high market value and whose nonvolatile compounds that have not yet been studied for use against cervical cancer cell lines. Here, we investigated the anticancer properties of organic extracts (ethyl acetate and methanol) prepared through the conventional Soxhlet (SOX) and Maceration (Mac) extraction methods against HeLa cells. Furthermore, spectrophotometer analysis via FTIR showed the presence of organic nonvolatile compounds in the dry leaf powder and which was confirmed through presence of functional groups.

However, HPLC and NMR spectral analysis of the organic extracts of *P.graveolens* (SOX and Mac) revealed the presence of secondary metabolites. We observed that HPLC spectral analysis of the ethyl acetate (SOX) extract revealed the presence of phenolic compounds at different retention times t_R ~28.39 min, t_R ~29.02 min, t_R ~ 30.96 min, t_R ~34.79 min, t_R ~43.93 min, and t_R ~ 63.1 min compared with those of the methanol extract. Moreover, H1 NMR spectral analysis of the ethyl acetate (SOX) extract confirmed the presence of hydrogen and this SOX extract of ethyl acetate significantly promoted the antiproliferative activity of the extract in HeLa cells. The most significant inhibition was measured by the IC_{50} values of the ethyl acetate (SOX) extract, which were 149.4+0.3 ug/mL and (Mac) 131.9 ±0.3 µg/mL This study provides evidence that P. *graveolens* organic extracts are promising potential compounds with significant antioxidant and anticancer effects against cervical cancer that suppress tumor growth.

Keywords: Natural drugs, FTIR, HPLC, *Pelargonium graveolens* L'Her. and Organic extracts

1. INTRODUCTION

Pelargonium graveolens L' Her. (*P. graveolens*) belongs to the Geraniaceae family, and is commonly known as rose geranium. *P.graveolens* has more than 280 species within the *Pelargonium* genus and is native to the southern parts of Africa. It is a small perennial shrub that grows in winter rainfall regions; it has a wide variety of growth habit and habitats. A mild climate with low humidity is ideal for its growth (Douglas 1969; Van DJJA, Vorster 1988; Prajapati et al. 2003). The plant has many medicinal properties i.g., antiseptic, anti-inflammatory, antimicrobial and anticancer properties and is used for treating skin problems and respiratory infections which can cause upper airway infection and fever, additionally; the plant helps in the immune system. In Particularly aromatic oils are complex combinations of phytochemicals such as terpenes, phenols, ketones and alcohols that are volatile in nature. These volatile compounds have anti-inflammatory, anti-biological and anticancer properties and nonvolatile compounds are natural antioxidants with anti-inflammatory properties. *Pelargonium graveolens* possess natural bioactive compounds such as 7-hydroxy-5, 6-dimethoxycoumarin, monomethyl ether, 7-O glucoside, scopoletin, the indole alkaloid elaeocarpidine and its 20-H isomer epielaeocarpidinein various parts of the plant and tartaric acid is one of the characteristic compounds of the genus *Pelargonium.*

The main purpose of this study is to identify the nonvolatile bioactive agent's the organic extract of *P.graveolens* to focus future research on pharmacological drug design.

2. MATERIALS AND METHODS

Ethyl acetate, methanol, formic acid , aceto nitrile, MTT salt, isopropanol, 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin, 100 µg/ streptomycin, trypsin, RPMI40 media, 0.1% DMSO and 45 µm syringe filters, were all purchased from Sigma Aldrich-India.(ATCC CCL-2) Human cervical carcinoma cell lines were purchased from the National Center for Cell Science, Pune, India.

2.1 Plant Materials

The fresh plant material *Pelargonium graveolens* for experimental analysis was collected from medicinal plant nursery, at the Central Institute of Medicinal and Aromatic Plants (CIMAP) Hyderabad, India. Authentication was performed based on the botanical characteristics of the plant material by

Dr. A. Shashikanth Taxonomist, Department of Botany, Osmania University, Hyderabad, Telangana, India.

2.2 Extraction

Pelargonium graveolens leaves with stem were collected from the Central Institute of Medicinal and Aromatic Plants (CIMAP) Hyderabad, India and washed under running tap water to remove adhering dust particles from the surface. After washing, the leaves were shade dried and fine powder was obtained for extraction analysis. Crude samples were extracted from the plant material with organic solvents by maceration and soxhlet extraction (Ennaifer Taroub Bouzaiene et al. 2018)

2.2.1 Maceration Extraction

The maceration method was carried out in a conical flask with coarsely powdered plant material soaked in the solvents for three days with normal shaking; such that the plant material was dissolve in the solvent. Crude extracts were used for the identification and characterization of natural compounds for pharmaceutical purpose (Zhang et al. 2018)

For the maceration study, 15 g of dry powder was weighed and submerged in 250 ml of individual organic solvents viz., methanol, and ethyl acetate in a flat bottom flask and kept for three days at room

temperature in an orbital shaker. On the third day, the organic phase was collected filtered and dried under reduced pressure at 40 °C on a rotary evaporator and after which Grade 1 filter paper was used. The resulting semi-solid crude extract was transferred into a small vial and the vials were stored at 4^0C for further experimental analysis.

2.2.2 Soxhlet Extraction

The Soxhlet extraction method is used for the isolation of compounds with limited solubility in solvent and is not suitable for isolating thermolabile compounds, as prolonged heating can lead to heat-sensitive compound For the experimental analysis degradation (Zhang 2018), 15 g of *Pelargonium graveolens* dry powder was placed in a thimble holder and 250 ml round bottom flask was connected to a soxhlet apparatus with an appropriate methanol and ethyl acetate. During Soxhlet extraction, cycles were run for two days at room temperature not more than the heating point of the solvent until a clear solution appeared in the thimble. Methanol and ethyl acetate filtrates were collected from the flask, and filtered through 45 μm syringe filters. The collected crude extracts were concentrated in a rotatory evaporator to obtain a semi-solid form at their respective boiling points. Semi-dried extracts were suspended in fresh ethyl acetate and methanol for further chromatographic analysis.

3.1 Anticancer Activity Assessment by MTTAssay

The anticancer activity of tumor cells was analysed by evaluating the capability of live cells, to reduce the yellow dye MTT to blue formazan as a product of live cells. According to Mosmann Tim et al., in the 1983 procedure, MTT salts pass into viable cells, Mitochondrion organelles contain NADPH enzymes, which reduce the MTT reagent to insoluble purple formazan, crystallized cells were solubilized by the organic isopropanol. MTT assay plate with sample was measured absorbance at 500-600 nm by using ELISA Reader (Mosmann 1983) The anticancer effects of *Pelargonium graveolens* ethyl acetate and methanol organic extracts were tested on HeLa (ATCC CCL-2) human cervical carcinoma cell lines. HeLa cell lines were grown in fresh medium in a T-25 flask containing 10% foetal bovine serum (FBS), to avoid bacterial contamination and 100 U/ml penicillin and 100 μg/ml streptomycin were added to sustain rapid development. The flasks were incubated in a CO2 incubator at room temperature and individual HeLa cells were collected from a T-25 flask by adding 2 ml of trypsin (trypsinization) or mechanically scraping method. Adhered cells were collected from T-25 flask by separating the cell lines by trypsinization into centrifuge tubes and centrifuging them at 500 rpm for 5 minutes. The cell pellet was resuspended in 2 ml of complete plain medium to a concentration of 1×10^4 cells/mL, 100 μL of HeLa cells were seeded in a 96-well microtitre plate, the plate was incubated at room temperature for one day, and the suspended cells were left to adhere to the wells. *P. graveolens* ethyl acetate and methanol (1 mg/mL) extracts were used for stock solution preparation and different dilutions of 0.5, 1, 1.5, 2, 2.5, and 3 μg /mL were used for each extract. After 24 hrs of incubation microtitre plates, the plates were monitored for confluence. RPMI 40 media were aspirated from the wells, fresh medium containing 100 μl of the organic extracts was added and 0.1% DMSO was added to the control wells. The microtitre plates were incubated further for 72 hrs, after incubation, 50 μL of MTT reagent was added and the mixture was incubated for another 2 hrs. Test samples were analysed in triplates and cisplatin was used as a positive control. After the incubation, the absorbance was read at 490 nm. The 50 %inhibitory concentration (IC50) values of the organic extracts were calculated via by regression analysis with Prism 6 software (Contreras-Ortiz et al, 2013).

The percentage of growth inhibition was determined by using the following formula,

Percent inhibition of cells = 100-[(At-Ab)/(Ac-Ab)]

At =test compound absorbance, sample and MTT solution

Ab = blank absorbance, DMSO

Ac = Control Absorbance; MTT Solution

The significance of differences in the inhibitory effects and IC50 values was calculated by reducing the absorbance by 50% for untreated cells.

4. Spectroscopic evaluation

4.1 Fourier Transform Infrared Spectrophotometer (FTIR)

On FTIR spectroscopy, 1 mg of *P.graveolens* dry powder was weighed and placed on the FTIR lens and the scan range was adjusted of 500 to 3500 cm with a resolution of 2 cm^{-1} and the study was carried out in the Department of Central Facilities for Research and Development, Osmania University, Hyderabad, India "(Ashok kumar R and Ramaswamy M 2014)

4.2 High-Performance Liquid Chromatography (HPLC)

HPLC is a versatile, reproducible chromatographic technique used to classify secondary metabolites in different plant organic samples. It has wide applications in various fields, active molecule identification and separation, and quantitative and qualitative estimation. HPLC chromatograms were obtained with a C-18 column (4.6 mm x 250 mm) filled with particles 5 μm in diameter. The HPLC instrument used was a SIL-20A (Japan) and "Prominence Autosampler". For the identification of substances, reciprocating pumps connected to a DGU-20A5 degasser and CBM-20A integrator, a UV-VIS detector, DAD SPD-M20A, and Program LC solution 1.22 SP1 were used. Individual 2 μL organic extracts were injected into a C18 column, and was set flow rate of 1 mL/min at 40°C. The column was run with two solvents, 20% solvent (A) formic acid: water 80% solvent (B) acetonitrile: water. Phenolic compounds were eluted at 280 nm and the gradient of solvent used was set at 5% B for 5 min, 10% B up for 10 min; 12% B for up to 16 min, 15% B for up to 25 min, and 100% B for 35 to 60 min. Functional compounds were identified based on the retention times and spectra of each compound peak in the sample relative to those of the standard phenolic compounds (Omoba et al. 2015).

4.3 NMR Spectroscopy Studies

NMR spectroscopy is a technique used to determine the purity, identity, and composition of the compounds in the same spectrum. The diverse compound movements of the proton as per their subatomic bonds inside the atom were measured by NMR spectroscopy (Bagavathi and Ramasamy 2012; Kimberly L.Colson 2015). A NMR study was carried out on Pelargonium *graveolens* organic extracts to detect and quantify the key metabolites known to exist in the extracts. In this process, all the samples were dissolved in 500 μl of DMSO-d6 solvent, vortexed for 1 min, sonicated for 5 min, and collected in NMR tubes. The NMR spectrum was run at 400 MHz with 15 scans and 300^{o}C on a Bruker Advance III spectrometer using a 1H-1H over Hauser effect spectroscopy (NEOSY) gradient pulse sequence for 5 min per sample and the use CDCl3 as the solvent and chemical shifts are represented in ppm. The NMR data were interpreted by Mnova NMR software on Windows10 PC (Anand S P, Jeyachandran R Nandagopalan 2011).

5. RESULTS

Preliminary analysis of organic extracts of *P.graveolens* of maceration and soxhlet methods revealed the

presence of secondary metabolites. Among the two organic solvent extracts, ethyl acetate had the most significant presence of carbohydrates, glycosides, alkaloids, sterols, flavonoids and lignin's compounds. These secondary metabolites have been proven to possess antioxidant and antimicrobial potential.

5.1 Fourier Transform Infrared Spectrophotometer The FTIR spectra obtained from the dry powder of *P.graveolens* are shown in Table1and Fig 1. The peak density ranged from 3303 to 611 cm^{-1}.The peak at 3303 cm^{-1} was attributed to the presence of alkynyl groups (C-H stretch). The peak at 2921 cm^{-1}~2852 cm^{-1} corresponds to the occurrence of alkyl compounds (C-H stretch). The bands at 1730 cm^{-1}~1442 cm^{-1} signify the presence of aromatic groups (C≡C bends) and the band at 1372 cm-^{1} is assigned to the presence of alkanes and alkyls (CH3 C-H bends). The peaks at 1259~1213 cm-1 indicated as aromatic and esters (O=C-O-C) and the peaks at 1033cm^{-1} were attributed to alkynes (≡C-H stretch) and those at 835~611cm^{-1} were attributed to alkyl halides (C-Br stretch).

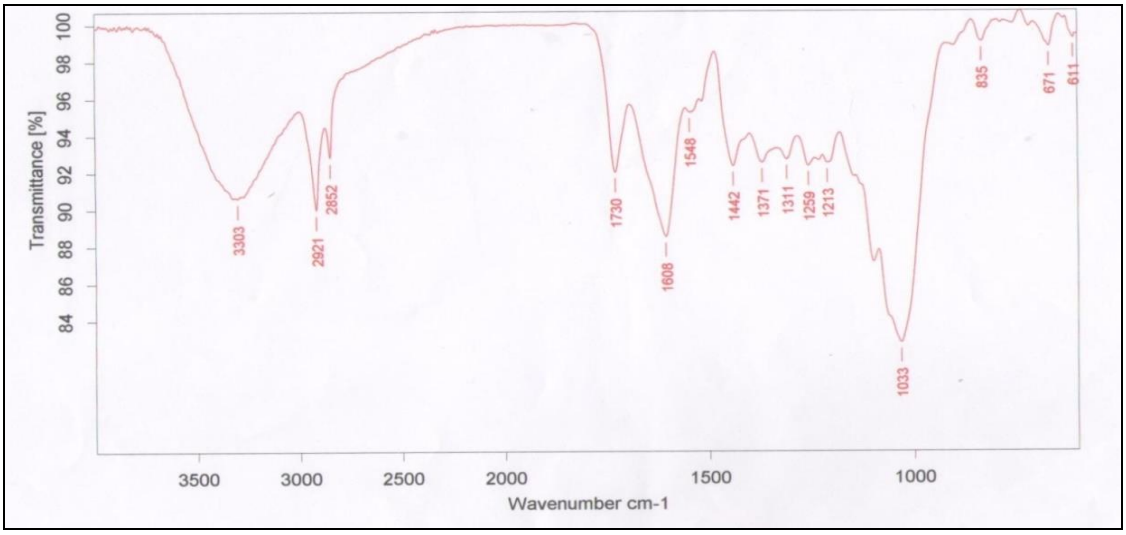

Figure 1: FTIR spectra of *P.graveolens* dry powder.

5.2 High-Performance Liquid Chromatography (HPLC)

The HPLC spectrum of the *P.graveolens* crude extracts showed elevated peaks in response to different organic solvents as shown in Figs 2, 3, 4, and 5. A Chromatogram of the organic extract of ethyl acetate showed 6 distinct peaks (1-6) at retention times of 28.39 (1), 29.02 (2), 30.96 (3), 34.79 (4), 43.93 (5), 63.12(6) min. The most isolated, pure and distinctive peaks were identified in the Soxhlet ethyl acetate extract of *P.graveolens* with reference to the standard compounds.

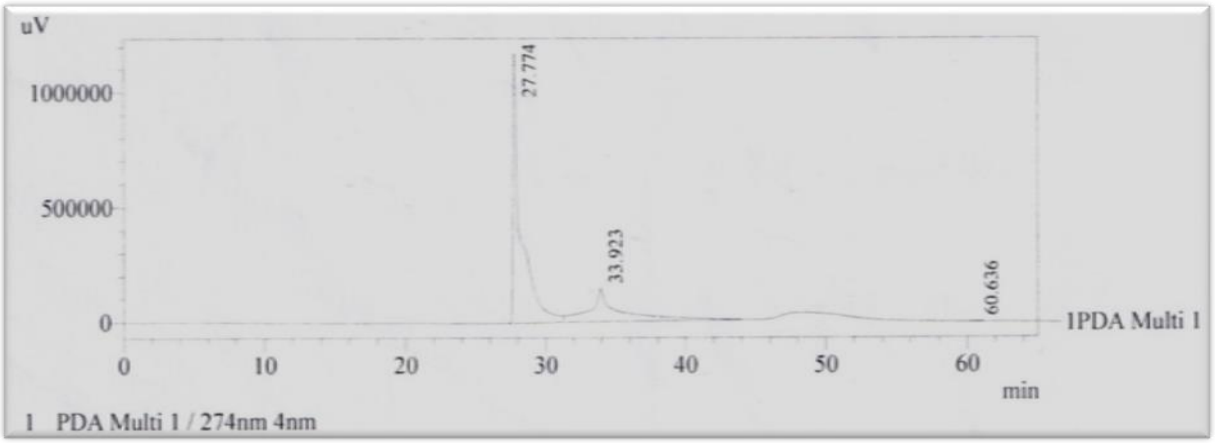

Figure 2: Analytical HPLC profile of the quercetin and kaempferol standards: An HPLC reverse phase Phenomenex C-18 column (4.6 mm × 250 mm packed with 5 µm diameters) was used. The two solvent systems used were isocratic 20% 0.1% (v/v) formic acid: water (A) and 80% (v/v) aceto nitrile: water (B). A total of 20 ul of 1mg/ml phenolic compounds was injected into the column and the chromatogram was collected at 280 nm for 60 min.

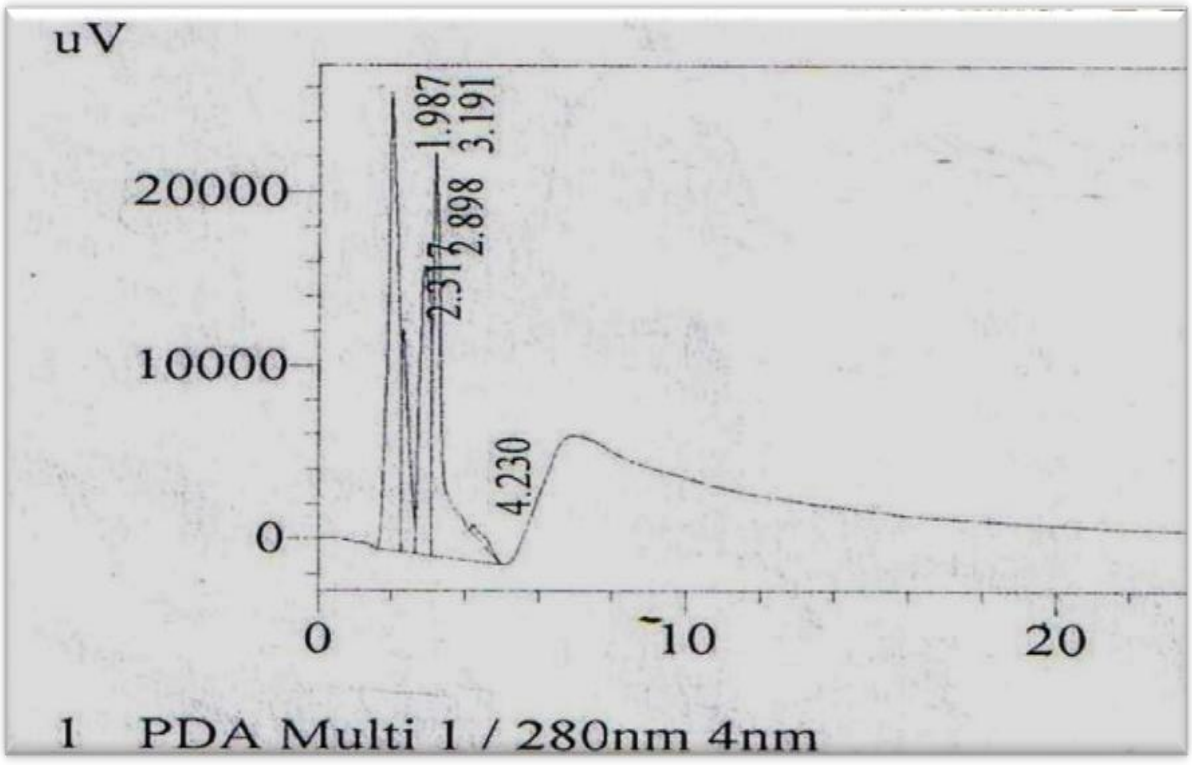

Figure 3: HPLC spectra of macerated methanolic extracts of *P.graveolens*: HPLC reverses Phenomenex C-18 column (4.6 mm × 250 mm) packed with 5 µm diameter particles with the mobile phases consisted of two solvents, 20% of 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile: water (B). Phenolic compounds 20 ul/mg were injected into the column and the chromatogram was read at 280 nm for 60 min.

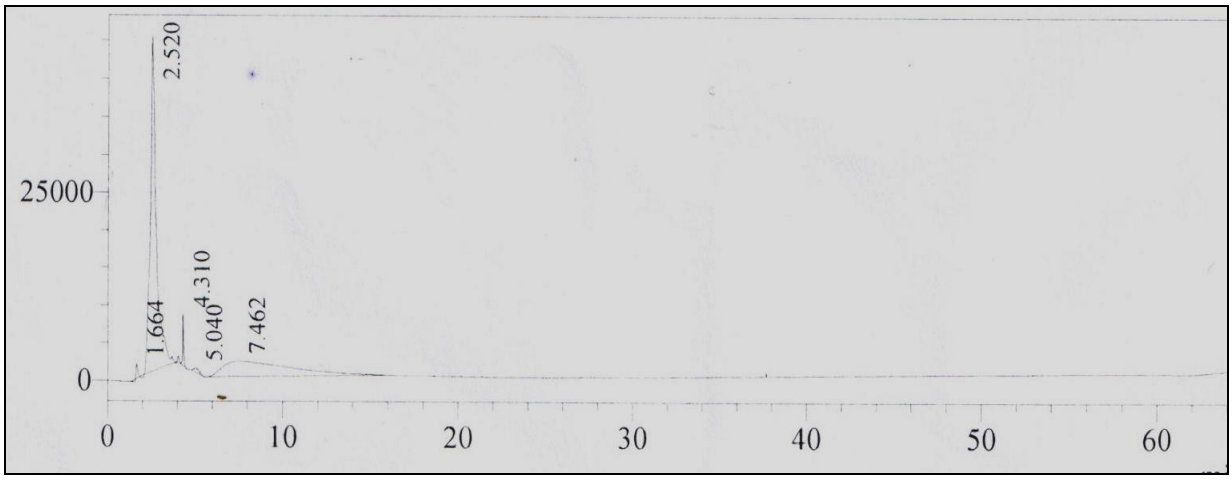

Figure 4: HPLC spectra of *P.graveolens* macerated ethyl acetate extracts: An HPLC reverse Phenomenex C-18 column (4.6 mm × 250 mm) was used to pack 5 µm diameter particles and the mobile phases consisted of two solvents, 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile: water (B), DAD UV detection of phenolic compounds was carried out at 280nm.

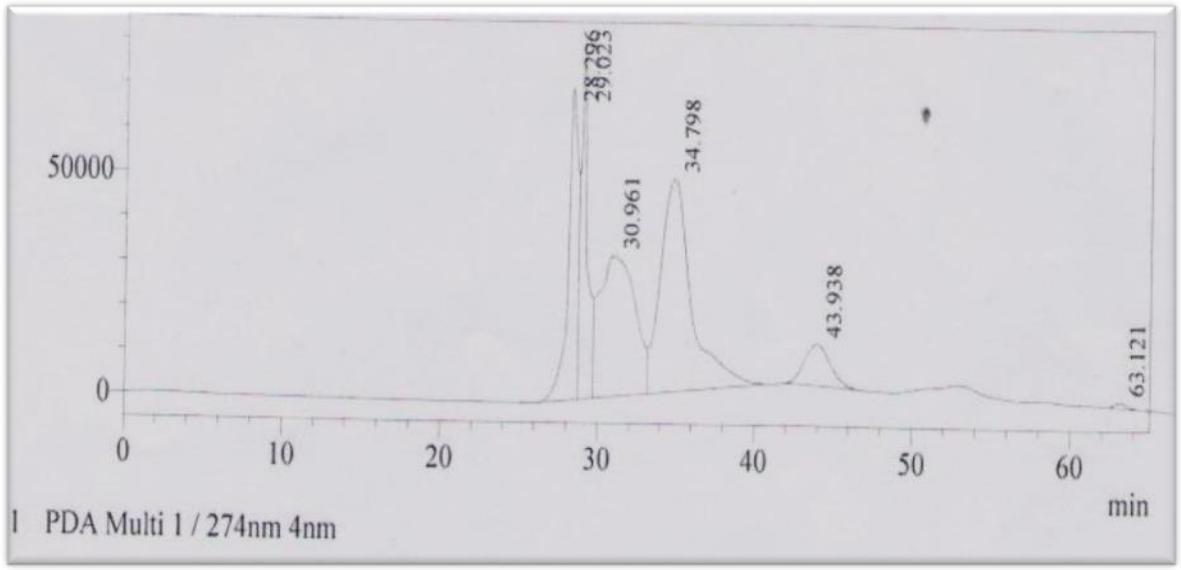

Figure 5: HPLC analyses of *P.graveolens* Soxhlet ethyl acetate extracts: A reverse phase Phenomenex C-18 column (4.6 mm × 250 mm) was used to pack 5 µm diameter particles, the mobile phases consisted of two solvents, 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile: water (B) at 280 nm.

5.3 Nuclear magnetic resonance (NMR) Spectroscopy

Proton ^{1}H NMR spectrum at 400 MHz,of the *P.graveolens* organic extracts was depicted in Figs 6, 7 and 8. The NMR spectrum for structure elucidation of each elevated peak showed the number of hydrogens present on each molecule with a double signal at δ 5.40 – 5.21 assigned to H-2 and H-5 and a doublet at δ 5.09 representing H-3 of the flavonoids. Doublets at δ 8.134 indicated the presence of aromatic rings in the flavonoid compounds. Fig 8 shows that the δ 8.09 -7.28 assigned to the H-2 proton and the δ 5.33 peak could be assigned to H-11. Doublet signals at δ 5.31-4.31 show at H-2 and δ3.00-3.70 which revealed H-28 and H-31 protons. The proton spectrum also showed a triplet signal at δ 0.92

H-5 and doublet signals at δ 0.88, which are identical to those of H-7. All these signals show the presence of aromatic compounds in the plant extracts. NMR analyses and structure elucidation of the secondary aromatic compounds in the three different organic extracts of *P.graveolens* revealed that the presence of hydrogen on each molecule corresponded to the peaks shown in Figs 8, 7 and 9 .

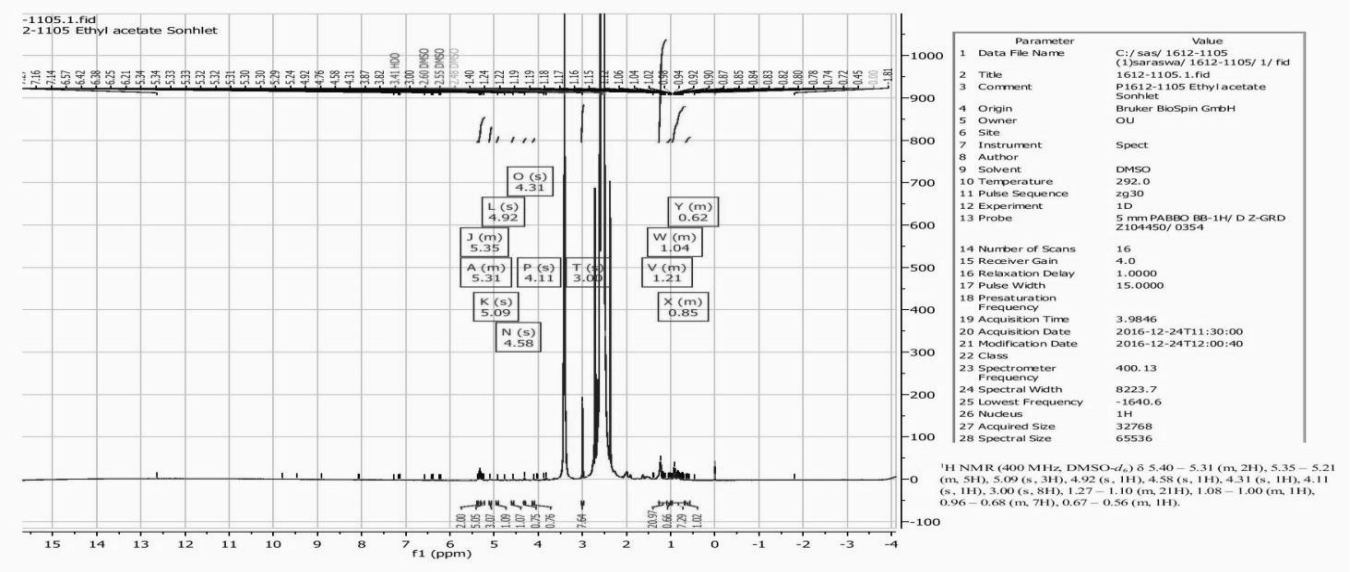

Figure 6 : ^{1}H NMR analyses of *P. graveolens* Soxhlet ethyl acetate extract *:* Soxhlet ethyl acetate extract ^{1}H NMR (400 MHz, DMSO-d_6) δ 5.40 – 5.31 (m, 2H), 5.35 – 5.21 (m, 5H), 5.09 (s, 3H), 4.92 (s, 1H), 4.58 (s, 1H), 4.31 (s, 1H), 4.11 (s, 1H), 3.00 (s, 8H), 1.27 – 1.10 (m, 21H), 1.08 – 1.00 (m, 1H), 0.96 – 0.68 (m, 7H), 0.67 – 0.56 (m, 1H). A value between δ 0.67 0 and δ 8.134 indicates the presence of aromatic rings in the compound.

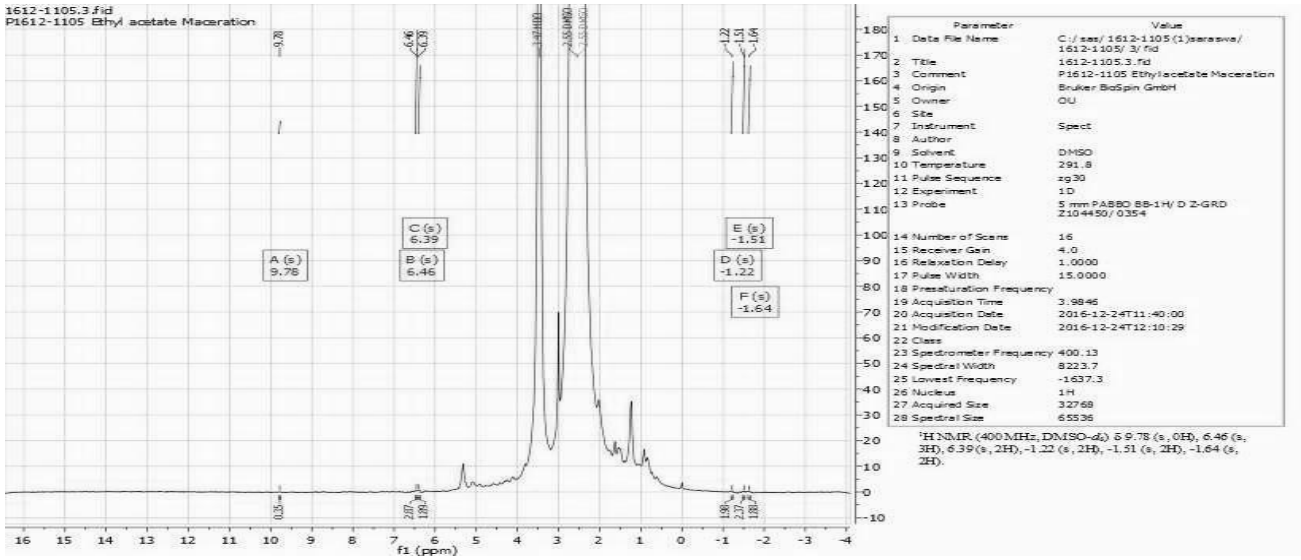

Figure 7: ^{1}H NMR analyses of *P. graveolens* macerated ethyl acetate extract: Macerated ethyl acetate ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.78 (s, 0H), 6.46 (s, 3H), 6.39 (s, 2H), -1.22 (s, 2H), -1.51 (s, 2H), and -1.64 (s, 2H).

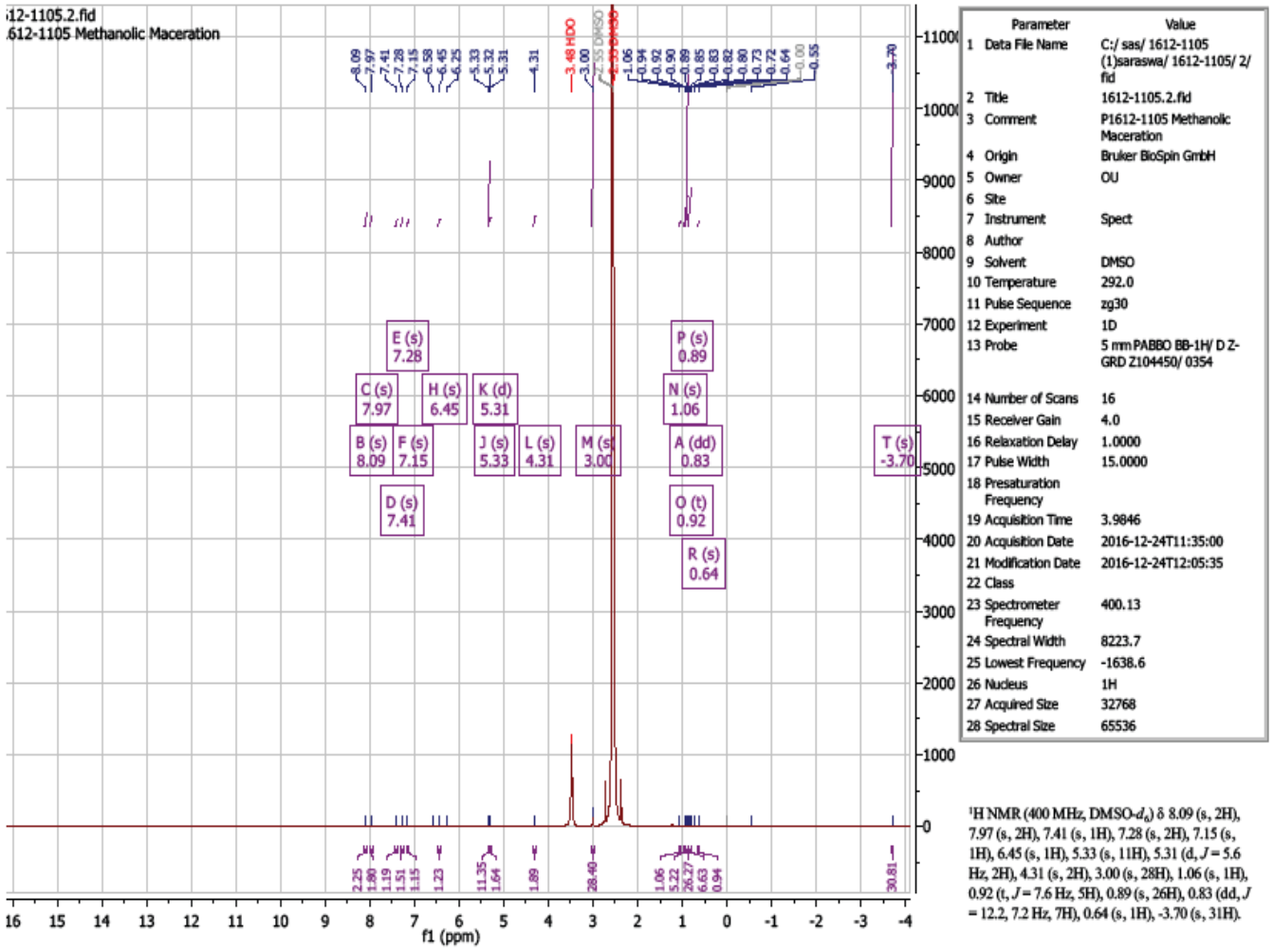

Figure 8: ^{1}H NMR spectra of *P. graveolens* maceration methanolic extracts : ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.09 (s, 2H), 7.97 (s, 2H), 7.41 (s, 1H), 7.28 (s, 2H), 7.15 (s, 1H), 6.45 (s, 1H), 5.33 (s, 11H), 5.31 (d, J = 5.6 Hz, 2H), 4.31 (s, 2H), 3.00 (s, 28H), 1.06 (s, 1H), 0.92 (t, J = 7.6 Hz, 5H), 0.89 (s, 26H), 0.83 (dd, J = 12.2, 7.2 Hz, 7H), 0.64 (s, 1H), and -3.70 (s, 31H).

5.4 Cytotoxic effects of *P.graveolens* extracts on HeLa cells

Cytotoxicity of the *P.graveolens* organic extracts was evaluated by using an MTT assay and the cell viability and percentage of inhibition were calculated for different concentrations of the organic extracts on cervical cancer cell lines. Cells were treated with increasing concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, and 300 µg/ml for 48 hrs for cell growth. A significant decrease or increase in the proliferation rate of HeLa cell was shown in comparison to that of the control group ($p<0.05$). the cytotoxicity of the organic extracts of *P. graveolens* and cell inhibition increased continuously depending on the duration of exposure from 48 hrs to 72 hrs. The range from 0.5 mg/ml to 3.0 mg /ml was dose dependent and cytotoxicity occurred after the first 24 hr and increased in parallel with the dose and duration of exposure. The percentage of the cytotoxic effect of the organic extracts on HeLa cells

are shown in Table 2. The data analyses were plotted in µg/ml on Graph pad prism 6 using log (inhibitor) versus response curve. The most effective concentration of extract was chosen based on the most significant IC $_{50}$ values. The results indicated that viability of HeLa cells treated with organic extracts at concentration ranging from 50-300 g/ml with reduction in cell viability in dose dependent manner, in which the cell viability decreased in soxhlet ethyl acetate extract. Ethyl acetate, acqu methanol, and citronellol exhibited significant cytotoxicity and IC_{50}values were calculated by Graph Pad prism 6 as presented in increasing order as ethyl acetate>methanol>citronellol with maximum valves of 183.5±0.12 µg/ml and 155±0.2 µg/ml respectively, Furthermore, the results revealed a significant correlation between the organic extracts and conventional methods. The morphology of the treated cells changed in an increasing dose-dependent manner as indicated by cell detachment, cell wall shrinkage, and death as observed under via inverted microscope. A morphological study revealed that the ethyl acetate Soxhlet extract showed greater inhibitory effects on the cells than the macerated methanol extract after 48 hrs of incubation.

Table 2: Proliferative effects of organic extracts and synthetic citronellol of on MTT treated cells *P. graveolens* analysis of the HeLa cell line

MTT assay for Cytotoxicity of HeLa Cells			
Concentration	**Ethyl acetate**	**Methanol**	**Citronellol**
50 µg/ml	57.006	38.96	38.209
100 µg/ml	66.028	43.472	48.735
150 µg/ml	85.577	70.539	52.494
200 µg/ml	89.336	70.765	56.254
250 µg/ml	96.855	86.329	72.035
300 µg/ml	106.63	100.615	87.081

Inhibitory percentage of cells treated with various concentrations of *P.graveolens* organic extract fo 72 hrs (SD = standard deviation).

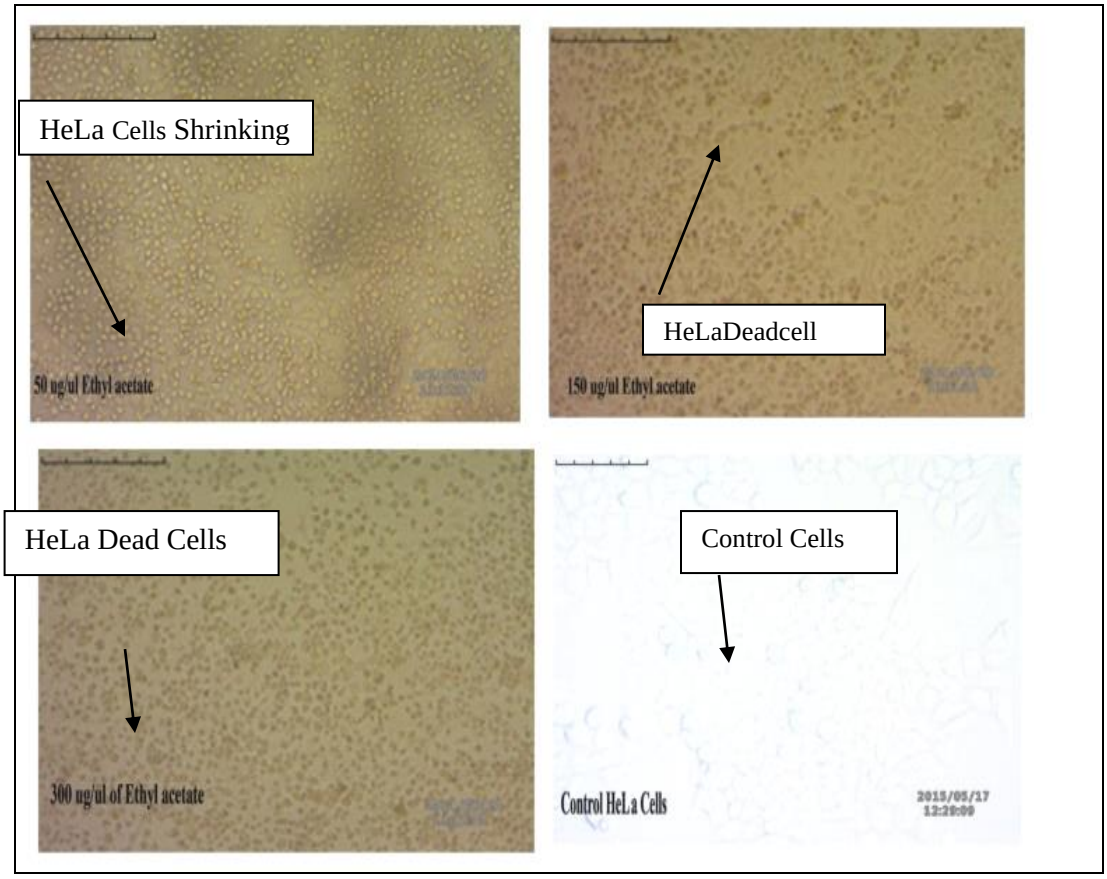

Figure 9: Comparison of cell morphology changes under an inverted microscope (200 X magnification) HeLa control cells a) HeLa cells treated with increasing concentrations of 50 µg/ml, 150µg/ml, and 300 µg/ml of soxhlet ethyl acetate extract at 48hr. 25 ug/ml of cisplatin was used as positive control and 10% DMSO was used as a negative control.

Table 3: ANOVA analysis of *P. graveolens* organic extracts on HeLa cell lines**.**

IC $_{50}$values of MTT Assay of *P. graveolens* organic extracts							
Synthetic extracts	**Ethyl acetate**	**Methanol**	**Citronellol**	**Control**	**Pearson r**	**P-value**	**Significant**
			183.5±0.12	**292.7±0.7**	**0.9272**	**0.0078**	**Yes****
Maceration Extract	**114.9±0.5**	**131.9±0.3**	**--**	**292.7±0.7**	**0.1087**	**0.8376**	**Ns**
Soxhlet Extract	**146.4±0.2**	**149.4±0.3**	**--**	**292.7±0.7**	**0.8462**	**0.0337**	**Yes ***

The IC_{50} values of the MTT assay were calculated at Mean±SD of the organic extract on HeLa cells P<0.005 according to the Tukey test (n = 3).∗∗∗: significant at the level p<0.01, ∗∗: significant at the

level $p<0.03$ and $*$: significant at the level $p<0.0$

6. DISCUSSION

According to preliminary analyses, the total yield of crude extract depends on factors such as the extraction method, solvent and temperature. During the study, the Soxhlet and maceration extractions methods were shown to be the most suitable for extracting and identify natural compounds from *P.graveolens.* The soxhlet method in which heating process directly affect the test sample and it results in temperature effects on plant samples, also has an impact on secondary metabolite content in the extracts (Roseiro 2013; Abubakar, 2020) These findings are repeated in many studies, which revealed that an increase in extraction temperature and time enhances the solubility of plant material (Masmoudi , 2008). The extracts will be in the dark greenish past and the major functional groups will be alcohols, phenols, alkanes, carboxylic acids, amides, alkenes, non-corrosive carbonyls, sweet-smelling amines, aromatics, alkynes, and alkyl halides with the presence of carbon hydrogen bond such as -C≡C, -CH3, -C-H, -O=C-O-C, and - ≡C-H as shown in Fig1 and Table 1. Similarly, the FTIR spectra of the organic extracts of *Calotropis* and *C.tora* plants revealed the presence of the secondary metabolites carbohydrates and flavonoids and the functional groups benzene and aldehyde. The presence of secondary metabolites in organic extracts is responsible for the antioxidant activity of plants (Mueen Ahmed 2005; Kareru 2008;Ying Zhang 2012). The present study limits ability of FTIR to identify function groups and in liquid extracts future studies are recommended.

Chromatography techniques are helpful for identifying the pure form of organic extracts. Chromatography of the *P.graveolens* Soxhlet ethyl acetate extract revealed elevated peaks corresponding to the standard compounds quercetin and kaempferol, and other peaks which could be esters or glycosides of phenol. HPLC studies of *P.graveolens* have reported the aliphatic amine 1, 3-dimethylamylamine (1, 3-DMAA) in volatile extracts (Thomas 2013; Lalli 2006), and quercetin, rutin, myricetin, and kaempferol in *pelargonium* species (Meena 2008). Flavonoid compounds were reported in the methanolic extract of *Couroupita guianensis.* Gallic acid and ferulic acid are found in *B.racemosa* (Kumaran K , Joel Karunakaran 2007), and rosmarinic acidis found in *Coleus aromaticus*. These bioactive compounds are derivatives of polyphenols and consist mainly of flavonols, flavones, catechins, and flavanones. They act as antioxidants and exhibit beneficial effects such as anti-inflammatory, antiviral, anti allergic and anticancer effects (Hussin 2009; Hillwell 1994). Previous research supports conventional extractions with significant difference in biological activities (Fraga ,1987; Hayouni 2007).

Cytotoxicity studies of plant organic extracts have been supported by many scientific works. Pazhanimuthu Annamalai, (2015) reported that the ethyl acetate extract of geranium species in HeLa and A673 cell lines had antiproliferative effects through the induction of apoptosis. *P. quercetorum* methanolic extract exhibited cytotoxic activity on A549, PC3, and H1299 lung cancer cell lines. *P. quercetorum* methanolic extracts induce apoptosis to cell death (Pazhanimuthu 2015; NazlihanAztopal. 2016). Similarly, F. *religiosa* aqueous and ethanolic bark extracts were cytotoxicity to SiHa and HeLa cervical cell lines. Natural compounds in crude extracts have effects on cell checkpoints and dead cells in cervical cancer cells (Choudhari , 2013). *E. alba* bioactive compounds exhibited the antiadipogenic and antidyslipidemic effects on 3T3-L1 and hMSC adipogenic cells. Abhishek Gupta et al, (2017)

reported that the *Vitisthunbergii var. taiwaniana* (VTT) ethyl acetate extract had apoptotic effects on the human prostate cancer cell line DU-145 by inducing mitochondrial changes in the cell cycle and apoptosis (Abhishek Gupta 2017; Lin C H 2018). Organic and aqueous extracts of the aerial parts of *Cyclotri chiumniveum* had cytotoxic effects on HeLa, MRC, and MCF-7 lines of cervical cancer cells. *Cyclotrichium* ethyl acetate and dichloromethane extracts induced cytotoxicity in MRC-5 and MCF-10 cells compared with other cell lines (Ozdemir A 2017). The *M. oleifera* ethyl acetate fraction exhibited potent anti-inflammatory activity on macrophages via suppression of the NF-κB signaling pathway (Arulselvan 2016). Thus, it has been proven that medicinal plant crude extract has cytotoxic effects on cell cycle arrest, inducing apoptosis, mitochondrial and morphological changes leading to cell death.

7. CONCLUSION

Qualitative and spectral analyses of the organic extracts of *P.graveolens* revealed distinct aromatic compounds. We evaluated the antimicrobial, anticancer, and antioxidant activities of the organic extracts identified the active compounds in organic extracts through FTIR, HPLC and NMR analysis.The spectral studies showed various characteristic absorption frequency peaks with functional groups of alkynyl, alkyl, alkanes, and aromatic. HeLa cells were treated with macerated and soxhlet extracts of ethyl acetate and methanol. Compared with the macerated methanol extract, which contained the positive control cisplatin, ethyl acetate had greater cytotoxic effects on the bacteria. The most significant difference in IC 50 was observed for synthetic citronella (183.5±0.12 ug/mL) compared with methanol (155±0.2 ug/ml). Thus, the results demonstrated that the pelargonium species have significant anticancer activity. Thus our finding showed that ethyl acetate of soxhlet extract exhibits a significant antimicrobial and anticancer activity. These spectral studies demonstrated the structure and nature of the bioactive compounds in *pelargonium graveolens*.

Declarations

Compliance with Ethical Standard

Not applicable

Consent for publication

I declare no consent for publication

Availability of data and material

The data will be available from the corresponding author upon reasonable request

Competing interests

I declare no conflicts of interest for this publication

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Author’s contributions

I Dr. J .Saraswathi contributed to the design of study, sample preparation and spectroscopy analysis and draft the manuscript for the publication.

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