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Phytochemical Profiling, Antioxidant and Anticancer Activity of *Nerium odorum* Plant Extracts

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Abstract: The aim of this study was to explore the phytochemical profile, *in vitro* free radical scavenging and *in vitro* cytotoxicity effects of ethyl acetate extract of *Nerium odorum*. The qualitative analysis of the phytochemicals of *Nerium odorum* was performed by Spectrophotometry method. Its antioxidant activity was analyzed by the method of DPPH- radical scavenging activity and bleaching of β -carotene. The *in vitro* cytotoxicity of ethyl acetate extract of *Nerium odorum* evaluated by WST-1 assay on normal and HT- 29 human colorectal adenocarcinoma cell line and MDA-MB231 metastatic mammary adenocarcinoma cell lines. Medicinal plants have been used for many human diseases such as cancer, heart diseases, arthritis, etc. Thus, in this study we have investigated the phytochemical profile of ethyl acetate extract of *Nerium odorum* such as preliminary screening, HPTLC quantification, GC-MS and FT-IR analysis. The ethyl acetate extract of *Nerium odorum* clearly possesses significant antioxidant potential due to its rich phytochemicals.

Keywords: Phytopharmacology, Cancer, Cell lines- HT-29, MDA-MB231, *Nerium odorum*, Metabolites

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Introduction

Phytochemicals are chemicals of plant origin (Breslin, 2017) which are produced by plants through primary or secondary metabolism (Molyneux, *et al.*, 2007). They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens, or predators (Molyneux *et al.*, 2007). Phytochemicals generally are regarded as compounds rather than essential nutrients

because proof of their possible health effects has not been well established (Heneman and Zidenberg-Cherr, 2008). Phytochemicals can be classified into major categories, such as carotenoids (Barbosa *et al.*, 2017) and polyphenols, which include phenolic acids, flavonoids, and stilbenes/lignans. Flavonoids can be further divided into groups based on their similar chemical structure, such as anthocyanins,

flavones, flavanones, and isoflavones, and flavanols (Babu and Ashok, 2021; Babu *et al.*, 2021). Flavanols further are classified as catechins, epicatechins, and proanthocyanidins. In total, there has been over 25,000 phytochemicals discovered and in most cases, these phytochemicals are concentrated in colourful parts of the plants like fruits, vegetables, nuts, legumes, and whole grains etc. (Erdman *et al.*, 2007).

In medicinal plants, phytochemicals such as alkaloids, tannins, saponins, flavonoids, phenols, steroids, carotenoids, etc. are present which have several disease prevention activities. These plant-derived chemical compounds play important preventive activities mainly anti-inflammatory, antidiabetic, antiaging, antimicrobial, antiparasitic, antidepressant, anticancer, antioxidant, and wound healing (Madhuri and Pandey, 2009). They also have great role in stress tolerance of plants and accumulation of many important bioactive compounds in fruits and vegetables. Flavonoids are the most common bioactive compounds found in medicinal plants. They have several preventive activities in human disease such as antimicrobial, antioxidant, anticancer, anti-inflammatory, and wound-healing capacity (Kris-Etherton *et al.*, 2002).

Anticarcinogenic flavonoids have been reported to be found in a number of fruits and vegetables. Apple and berries found to have cardio-protective properties and showed positive impact on blood pressure (Kaur and Kapoor, 2001). Anthocyanins are the flavonoid constituents abundant in cell vacuole responsible for pigmentation in flowers, fruits, and vegetables and are produced generally during plant under environmental stress. *In vitro* studies showed antioxidative activities of anthocyanins in cell culture systems such as colon, liver, breast, leukemic cell, and keratinocytes (Bueno *et al.*, 2012). Carotenoids are considered as the potential natural antioxidant found in fruits and vegetables. They include xanthophyll and carotenes having

scavenging of peroxy radical. Lycopene is common in tomato and berries, while β -carotenes are orange-colored carotenoids abundant in yellow-orange and dark-green leafy vegetables (Tapiero *et al.*, 2004).

Oxygen is an indispensable element for life. However, under certain conditions it can have serious deleterious effects on the body. Oxygen can produce highly reactive compounds called reactive oxygen species (ROS) (Bhattacharya, 2015). Many such reactive species are free radicals containing an unpaired electron. They have a tendency to either accept or donate an electron and are, therefore, unstable and highly reactive. Hydroxyl radical (OH $\cdot$), the superoxide radical (O $_2^{\cdot-}$), the nitric oxide radical (NO $\cdot$) and the lipid peroxy radical (LOO $\cdot$) are amongst the most important oxygen-containing radicals formed in the body. Free radicals can attack a number of macro-molecules including lipids, proteins and DNA resulting in the cellular damage. Free radicals and other ROS are derived either from the endogenous metabolic processes in the human body or from external sources (Kehrer, 1993). The former includes mitochondrial respiration, peroxisomal metabolism, phagocyte activity, arachidonate pathways, inflammation, ischaemia, exercise and reactions involving iron and other transition metals. External sources include exposure to radiation, ozone, cigarette smoke, air pollutant and industrial chemicals. Antioxidants are molecules that can safely react with free radicals to neutralize or terminate the chain reaction before vital molecules are damaged (Heffner and Repine, 1989).

Oxidative stress is an imbalance between the production of ROS and the defense systems to readily detoxify them. In human, oxidative damage and free radicals are associated with a number of diseases including atherosclerosis, Alzheimer's disease, cancer, ocular disease, diabetes, rheumatoid arthritis and motor neuron disease. Many studies have shown the benefit of antioxidants in the prevention or delaying the

course of these diseases. Amongst these antioxidants dietary supplements such as vitamin E, vitamin C and beta-carotene are widely used (Hajhashemi *et al.*, 2010).

The WST-1 Cell Proliferation Assay is a colorimetric assay that is based on the cleavage of a tetrazolium salt, MTS, by mitochondrial dehydrogenases to form formazan in viable cells. The greater the number of viable, metabolically active cells, the greater the amount of formazan product produced following the addition of WST-1. By detection of the formazan level in the cells, we can quantify the cell number (Quent *et al.*, 2010). The assay can be used not only for quantifying cell proliferation, but cytotoxicity can also be measured. WST-1 can be used to assay either adherent or suspension cells. The WST-1 assay is very sensitive; it can detect 500 to 50,000 cells in a single well of a 96-well plate. Another advantage of the WST-1 assay is that there is no need to wash or harvest cells for the assay. It is not needed to remove culture media or wash with PBS for this assay (Kamiloglu *et al.*, 2020).

Another advantage of the WST-1 assay is that the color change (to yellow) is visible to the naked eye. Absorbance is then measured at 450 nm versus a 650 nm reference by using a plate reader to compare experimental samples to controls. Spectra MAX 190 microplate reader is used and SoftMax Pro software from Molecular Devices. Results are analyzed by exporting the data to MS Excel and expressing the mean $\pm$ SD for each sample. This assay is easy to use and generate reliable results (Xie *et al.*, 2007).

The underlying cause of cancer is thought to damage DNA, much of which is oxidative in nature. These oxidative processes, the mechanisms of which not fully understood, occur during the promotional stage of carcinogenesis. Therefore, it is plausible that antioxidants may be able to interfere with the metabolic activation of chemical carcinogens, cause regression of pre-malignant lesions or inhibit their development into cancer. Evidence from basic research and observational

epidemiologic studies suggest that individuals with high intakes of fruits and vegetables experience lower risks of developing cancer (Valavanidis *et al.*, 2013). Although there are many compounds in fruits and vegetables that may potentially influence cancer risk, it is generally assumed that certain antioxidants such as vitamin E, Vitamin C and beta-carotene may be responsible for the lower cancer rates. However, the few randomized trials of vitamin E, vitamin C or beta-carotene supplementation show no overall benefits; some even suggest harm (Rietjens *et al.*, 2002).

Cytotoxicity studies are a useful initial step in determining the potential toxicity of a test substance, including plant extracts or biologically active compounds isolated from plants. Minimal to no toxicity is essential for the successful development of a pharmaceutical or cosmetic preparation and in this regard, cellular toxicity studies play a crucial role. The concept of basal cytotoxicity, where deleterious effects are noted on structures and functions common to all human cells, is relevant when considering the relationship between acute toxicity and cytotoxicity (McGaw *et al.*, 2014).

These studies not only save human lives and test animals, but they save the time and resources to be spared on a test molecule which is a complete failure having no *in vitro* safety. As scientific and medical studies are getting more expensive over the recent decades, most of the universities, research institutes are underfinanced, the importance of a certain method's price is greater than ever before. Animal experiments are expensive and the administrative burden is overwhelming, so they are only used when no other test is suitable. Also, every year new plants and their respective metabolites are described and through the various methods of chemistry, new molecules are synthesized, and even an older compound might be re-evaluated for a new indication (Corsello *et al.*, 2020).

Secondary cell lines are ideal for the screening

of the enormous amount of test subjects. If the right cell line is chosen, not just the cytotoxicity, but the biological activity of the chosen material can be measured. In short time, multiple experiments can be carried out, with high reproducibility in the same test system. If we use more than one method, with different signaling mechanisms and different cell lines, we will have a more complex view on the *in vitro* toxicity data (Pagliero *et al.*, 2016). This means more information when planning the *in vivo* experiments. Generally, it can be said, that if a compound proves to be non-toxic, then *in vivo* it will be tolerable. If it is moderately toxic, there is a chance that the 3D structure and the different cell types of an organ or the human body can effectively recover from the cytotoxic damage or the damage will only be minimal (Bácskay *et al.*, 2018).

The aim of this study was to explore the phytochemical profile, *in vitro* free radical scavenging and *in vitro* cytotoxicity effects of ethyl acetate extract of *Nerium odorum*. The qualitative analysis of the phytocompounds of *Nerium odorum* was performed by Spectrophotometry method. Its antioxidant activity was analyzed by the method of DPPH- radical scavenging activity and bleaching of β -carotene. The *in vitro* cytotoxicity of ethyl acetate extract of *Nerium odorum* evaluated by WST-1 assay on normal and HT- 29 human colorectal adenocarcinoma cell line and MDA-MB231 metastatic mammary adenocarcinoma cell lines.

Materials and Methods

Collection of Sample:

The medicinal plant used for the experiment was the leaves of *Nerium odorum*. The plant parts were identified by Dr. Shashikala, Central Research Institute Anna Nagar, Chennai, India and the respective herbariums were submitted there by (Sivagnanam *et al.*, 2012).

Preparation of extract:

500 g of dried powder of *Nerium odorum* leaves

was packed in separate round bottom flask for sample extraction using different solvents namely ethanol, methanol, chloroform, ethyl acetate and water. The extraction was conducted with 750 ml of each solvent for a period of 24 h. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts were stored in refrigerator.

Phytochemical Analysis:

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature (Ralte, 2014).

GC-MS:

GC-MS analysis of the ethyl acetate extract of *Nerium odorum* was carried out using a Clarus 500 Perkin-Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo Mass Gold Perkin-Elmer Turbo Mass 5.1 spectrometer with an elite - 1 (100% Dimethyl poly siloxane), 30 m $\times$ 0.25 mm ID $\times$ 1 μ m of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system, and was operated in electron impact mode with ionization energy of 70 eV. The instrument was set to an initial temperature of 110 C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature rose up to 280 C, at the rate of an increase of 5 C min⁻¹, and maintained for 9 min. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 ml was employed (split ratio of 10:1). The injector temperature was maintained at 250 C, the ion-source temperature was 200 C, the oven temperature was programmed at 110 C (isothermal for 2 min), with an increase of 100 C min⁻¹ to 200 C, then 5 C min⁻¹ to 280 C, ending with a 9 min isothermal at 280 C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The samples were

injected in split mode as 10:1. Mass spectral scan range was set at 45 to 450 (m/z). The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.2 (Jasim *et al.*, 2015).

FT-IR:

FTIR spectrophotometer was used to identify the characteristic functional groups in ethyl acetate extract of *Nerium odorum*. Small quantity (<10 mg) of extract was taken in CaF₂ vessel and placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Shimadzu 8300 FT-IR spectrophotometer at ambient temperature. Background correction was made by taking IR spectrum of de-ionized water as the reference in identical condition. The sample was scanned from 400 to 4,000 cm⁻¹ for 16 times to increase the signal to noise ratio.

HPTLC:

The supernatant solutions were used as test solution for HPTLC analysis. 1.5 µl of test solution and respective quantity of standard solution for the respective component was loaded as 5 mm band length in the 3 x 10 Silica gel 60F254 TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed in the respective mobile phase up to 80-90 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at White light, UV 254 nm and UV366 nm. The developed plate was sprayed with respective spray reagent and dried at 100 C in Hot air oven. The plate was photo-documented in Day light mode using Photodocumentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the plate was fixed in scanner stage and scanning was done at UV range of 254 nm to 500 nm according to the requirement of the different

components. The Peak table, Peak display and Peak densitogram were noted.

Anti-oxidant analysis:

DPPH radical scavenging activity:

The hydrogen atoms or electrons donation ability of the plant extracts were measured using the bleaching of a purple-colored methanol solution of DPPH (Bácskay *et al.*, 2018). Aqueous and ethanolic extracts were tested at concentrations ranging from 0.312 5 mg/ml to 10 mg/ml. Briefly, 150 µL of each concentration was deposited in the wells of a 96-well microtiter plate and then 50 µL of 1 mM DPPH were added to each well. The microplates were kept at room temperature in the dark for 30 min (Rajasekar *et al.*, 2020b). The absorbance was then measured at 517 nm. BHT, a standard antioxidant, was used as a positive control. The percentage of DPPH trapping activity was calculated as follow:

$$\% \text{ Inhibition} = A \text{ control} - A \text{ sample} / A \text{ control} \times 100\%$$

Where A stands for absorbance, control represents purple-colored methanol solution of DPPH and sample is the plant extract tested at different concentrations. The concentration providing 50% inhibition (IC₅₀) calculated from the dose response graph.

Bleaching of β-carotene:

β-carotene bleaching assay was carried out according to the method reported by Lu *et al.* (2015). Briefly, 0.14 mg of β-carotene was dissolved in 0.7 mL of chloroform. Then, 200 µl of the mixture was kept into a flask containing 1.4 mg of linoleic acid and 14 mg of Tween 40, the solution was vigorously stirred and the chloroform was then removed by incubation of the solution at 50 C for 2 h. After the chloroform had evaporated, distilled water was added to form an emulsion and 200 µl of this emulsion were added to the wells of a micro titration plate supplemented with 8 µl of different concentrations of the extracts ranging from 0.312 mg/ml to 10 mg/ml. The absorbance of the β-

carotene emulsion was measured at 470 nm, immediately (T0) and after incubation at 50 C for 120 min (T120). The antioxidant activity is expressed as a percentage inhibition with reference to the control according to the following equation:

$$\% \text{ Inhibition} = \frac{\text{A sample at T120} - \text{A control at T120}}{\text{A sample at T0} - \text{A control at T0}} \times 100\%$$

Cytotoxicity analysis:

Cell Culture:

Cell lines used in this study included MDA-MB-231, a metastatic mammary adenocarcinoma and HT-29, a human colorectal adenocarcinoma cell line. Cancerous cell lines were cultured in DMEM medium supplemented with 1% glutamine (Gibco), 10% fetal calf serum (Gibco) and 1 % of mixture of streptomycin/penicillin. Cells were maintained at 37 C with 5% CO₂ and 95% humidity. The cells were fed until confluent and expanded by trypsinisation, and then sub-cultured at lower numbers in new culture flasks. The viability was checked by trypan blue and if it was more than 95%, the experiments were performed (Rajasekar *et al.*, 2020 a, Ashok and Babu, 2021).

Cell proliferation (WST-1) assay:

Cell viability was estimated on the basis of mitochondrial metabolic activity using WST-1 which is disodium mono{4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-tetrazol]-3-ium-5-yl] benzene-1,3- disulfonate} (Erdman *et al.*, 2007). Exponentially growing MDA- MB-231 and HT-29 cells were seeded on 96-well microplates at a density of 8000 cells per well. After 24 h, the culture medium was replaced by an experimental one with different concentrations of the extracts (initially dissolved in DMSO). Narrow range concentrations tested in this study varied from 0.75 µg/ml to 20 µg/ml, obtained after diluting stock solutions with media so that the final concentrations of DMSO did not exceed 0.05%. Each concentration/assay was performed in duplicate. The cells were then re-incubated for 72 h. Then, 100 µl of the medium were aspirated from

each well, 10 µl of WST-1 reagent was added and the plate was re-incubated for further 4 h.

Results and Discussion

Phytochemical analysis:

Table 1 shows the phytochemical constituents of ethanolic, methanolic, chloroform, ethyl acetate and aqueous extracts of *Nerium odorum*. Phytochemical screening of all the five extracts viz. ethanol, methanol, chloroform, ethyl acetate and aqueous, showed the presence of flavonoids. Alkaloids were also confirmed in all the extracts of *Nerium odorum*. Chloroform showed the presence of tri-terpenoids while ethanol, methanol, ethyl acetate and aqueous showed negative results. Saponins were found in aqueous extract while rest extracts viz. ethanol, methanol, ethyl acetate and chloroform showed negative result for saponins. Tannins were present in chloroform, ethyl acetate and aqueous extracts while ethanol and methanol showed negative result. Ethanol, methanol and ethyl acetate extract showed the presence of triple sugar while chloroform and aqueous extract showed negative result. Amino acids were absent in all the five extracts. Ethanol, methanol and aqueous extract showed the presence of anthraquinones while chloroform and ethyl acetate showed the negative result. Steroids are present in ethanolic, methanolic and aqueous extracts while absent in chloroformic and ethyl acetate extracts. Proteins were absent in all the five extracts. Cardiac glycosides were found in only ethyl acetate extract while rest four extracts showed negative.

GC-MS:

GC-MS analysis of alkaloid compound clearly showed the presence of fifteen compounds. The alkaloid compound, formula, molecular weight and exact mass are as shown in Table 2. The GC-MS chromatogram of the 15 peaks of the compounds detected are shown in Figure 1. Chromatogram GC-MS analysis of the ethyl acetate extract of *Nerium odorum* showed the presence of

Table1 1: Preliminary Phytochemical Analysis

S. No.	Phytocompounds	Ethanol	Methanol	Chloroform	Ethyl Acetate	Water
1	Flavanoids	+	+	+	+	+
2	Alkaloids	-	+	+	+	+
3	Tri-Terpenoids	-	-	+	-	-
4	Saponins	-	-	-	-	+
5	Tannins	-	-	+	+	+
6	Triple Sugar	+	+	-	+	-
7	Amino acids	+	+	-	-	+
8	Antroquinones	+	+	-	-	+
9	Steroids	+	+	-	-	+
10	Proteins	-	-	-	-	-
11	Cardiac Glycoside	-	-	-	+	+

Table 2: Compounds present in the ethyl acetate extract of *Nerium odorum*

Compound	Rt (min)	Formula	Molecular weight	Exact mass
2-Cyclopenten-1-one, 2- hydroxy-3-methyl	4.952	C ₆ H ₈ O ₂	112	112.0524297
5-Hydrooxy methylfurfural	6.686	C ₆ H ₆ O ₃	126	126.031694
β-d-allopyranoside, methyle 6-dioxy-2-o methyl	9.043	C ₈ H ₁₆ O ₅	192	192.099773
2-Cyclohexen-1- one, 4(hydroxyl butyle)- 3.5.5-trimethyl	11.904	C ₁₃ H ₂₂ O ₂	210	210.16198
3-Eicosene	13.449	C ₂₀ H ₄₀	280	280.313002
1-Monolinoleoylglycerol Trimethylsilyl ether	15.938	C ₂₇ H ₅₄ O ₄ Si ₂	498	498.356064
9.12.15-Octadecatrienoic acid,	17.329	C ₂₇ H ₅₂ O ₄ Si ₂	496	496.340414
2,3bis[trimethylsilyl]oxy] propyl ester Octadecane, 3-ethyl-5-(2-ethylbutyl)	19.818	C ₂₆ H ₅₄	366	366.422552

fifteen major peaks and the components corresponding to the peaks were determined as follows. The first set up peak was determined to be 5-hydrooxy methylfurfural, β-d- allopyranoside. The second peak indicated to be methyle 6-dioxy-2-o methyl. The next peaks was considered to be cycloheptasiloxane, tetradecamethyle, cycloocta-

siloxane, hexadecamethyl, cyclo nonasiloxane, octadecamethyl, cyclodecasiloxane, eicosamethyl, 2- cyclopenten-1-one, 2-hydroxy-3-methyl, 9.12.15-octadecatrienoic acid 2,3 bis [trimethylsilyl] oxy] propylester, octadecane, 3-ethyl-5-(2-ethylbutyl), 1-monolinoleoyl glycerol trimethylsilyl ether, 1.1.3.3.5.5.7.7.9.9-

Fig. 1: GC-MS profile of ethyl acetate extract of *Nerium odorum*.

Fig. 2: Fourier-Transform Infrared Spectra of *Nerium odorum* extract.

decamethyl-9-(2-methyl propoxy) pentasil oxane, 2-cyclohexen-1-one, 4 - (hydroxybutyle) - 3.5.5-trimethyl, octasil oxane, 1.1.3.3. 5.5.7.7.9.9.11.11.13.13.15.15-hexadecamethyl and 3-eicosene. The identified phyto-compounds have the property of anti-oxidant and antimicrobial activities.

The IR spectrum (Fig. 2) of ethyl acetate extract of *Nerium odorum* displayed different peaks (Table 3) corresponding to different functional groups (Dey and Chaudhuri, 2016). The 2,923.9 cm^{-1} peak was formed primarily due to

the presence of methylene groups. Presence of two peaks, one close to 2,330 cm^{-1} and another close to 667 cm^{-1} may correspond to the presence of ketone group such as in some aliphatic moieties. Presence of alkanes was detected by the two peaks at 1,460 cm^{-1} and 1,379 cm^{-1} . Peak shifts in FTIR analysis are very common phenomenon. Some of the functional groups may thus, be affected by peak shift. The peak at 1,379.0 cm^{-1} may correspond to aromatic amines indicating C-N stretching (Erdman *et al.*, 2007). The peak at 2,856.4 cm^{-1} may be formed due to the

Table 3: Fourier-Transform Infrared Spectroscopy peak values of *Nerium odorum* extract

Wave number (cm ⁻¹)	Type of Bond	Functional Groups	Types of Vibrations	Intensities
2,923.9	CH ₂ -O	Alkane (C-H) Aliphatic	Stretching	Strong
2,856.4	CH-pyrrol	Alkane (C-H)	Stretching	Strong
2,362.6	C=C	-	Stretching	-
2,333.7	C=O	Aliphatic Ketone	-	-
1,560.3	N-O N-H	Nitro Amide	Stretch Bending	Strong -
1,460.0	C=C CH ₃	Aromatic Alkane (C-H)	Stretching -	Medium-weak -
1,379.0	-C-H N-	Alkane Nitro	Bending Stretching	Variable Strong
1,280.6	C-O	Acid	Stretching	Strong
754.1	=C-H	Alkene	Bending	Strong
675.0	=C-H	Alkene	Bending	Strong
418.5	N-H	Amide	Bending	-

Fig. 3: Baseline display (Scanned at 254nm).

presence of carboxylic acids (Molyneux *et al.*, 2007).

Alkaloids are heterogeneous group of compounds which contain one or more nitrogen atom in acyclic system. These are widely used for medicinal purposes which have positive and negative effects even on human beings. One type of alkaloid was found in *Nerium odorum*. The HPTLC densitometric chromatogram for alkaloids is represented in Figures 3, 4, and 5.

Anti-oxidant activity:

DPPH radical scavenging activity:

In this study, *Nerium odorum* ethyl acetate and aqueous extracts were investigated for their antioxidant activity with DPPH scavenging assay (Table 4). Results of the antioxidant power of *Nerium odorum* extracts compared to the standard product were reported. The aqueous extract showed a high antioxidant activity and all the used concentrations had a powerful effect (above 50%),

Fig. 4: Peak densitogram display (Scanned at 254nm).

Fig. 5: 3D Display of all tracks.

Table 4 : Scavenging activities of different concentrations of *Nerium odorum* ethyl acetate and aqueous extracts on the DPPH radical (%)

Extract (mg/ml)	1.25	2.5	5	10	P	F
Aqueous extract	90.26±1.66	91.54±2.05	92.67±1.21	93.17±0.62	0.10	2.21
Ethyl acetate	73.50±1.09	84.74±0.46	87.75±2.18	87.25±0.46	0.05	1.95
BHT	93.92±1.39	94.18±1.73	95.18±1.23	98.01±3.45	-	-

similar to that obtained with BHT, used as a reference molecule (F=2.21, P=0.10). However, the ethyl acetate extract showed a dose-dependent activity giving an IC₅₀ of (2.200 ± 0.014) mg/ml. Levene test analysis showed a significant difference between ethyl acetate extract and BHT

activities (IC₅₀=3.48 µg/ml) (P<0.05).

Plants are the richest resource of drugs. Traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediate and chemical entities for synthetic drugs are directly

or indirectly dependent on plants. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization (Suresh and Sisodia, 2018). The earliest mention of medicinal use of plants in Hindu culture is found in “Rigveda”, which is said to have been written between 4500 -1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing. Medicinal plants are a source of great economic value all over the world (Prasad, 1949).

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents (Sumit and Geetika, 2012). Among the 7,000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are found in India. Unfortunately, only few of them are used for their medicinal value. About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha (Giday *et al.*, 2003).

However, the ethno pharmacologists, botanists, microbiologists and natural-product chemists world over today, is constantly still in search of medicinal efficacy of plants and their phytochemicals, since the reported data so far available on plants are comparatively meager before the vast number of plant population. The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue.

Conclusion

Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA. This Herbal and natural products have been used in folk medicine for centuries through-out the world, but there are

relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, this coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs.

References

- Ashok K and Babu M. (2021) Larvicidal activity of grass weed *Setaria viridis* extracts. Intern J Zool Invest. 7(2): 596-599.
- Babu M and Ashok K. (2021) Larvicidal activity of onion (*Allium cepa*) peel extracts against *Anopheles stephensi*. Intern J Zool Invest. 7(2): 600-602.
- Babu M, Ashok K, Jula V and Mullai NK. (2021) A review on traditional medicinal plants with anti diabetic properties. Linguistics Culture Rev. 5(S1): 1244-1251.
- Bácskay I, Nemes D, Fenyvesi F, Váradi J, Vasvári G, Fehér P, Vecsernyes M and Ujhelyi Z. (2018) Role of cytotoxicity experiments in pharmaceutical development. doi:10.5772/INTECHOPEN.72539.
- Barbosa A, Silveira GD, deMenezes I, Neto J, Bitencurt J, Estavam CS, de Lima Ado C, Thomazzi SM, Guimarães AG, Quintans LJ Jr and dos Santos MR. (2013) Antidiabetic effect of the *Chrysobalanus icaco* L. aqueous extract in rats. J Med Food. 16(6): 538-543.
- Bhattacharya S. (2015) Reactive oxygen species and cellular defense system. In: Free radicals in human health and disease, Springer, New Delhi, pp. 17-29.
- Breslin A. (2017). The chemical composition of green plants. Sciencing, Leaf Group Ltd.
- Bueno JM, Sáez-Plaza P, Ramos-Escudero F, Jiménez AM, Fett R and Asuero AG. (2012) Analysis and antioxidant capacity of anthocyanin pigments. Part II: Chemical structure, color, and intake of anthocyanins. Critical Rev Anal Chem. 42(2): 126-151.
- Corsello SM, Nagari RT, Spangler RD, Rossen J, Kocak M, Bryan JG, Humeidi R, Peck D, Wu X, Tang AA, Wang VM, Bender SA, Lemire E, Narayan R, Montgomery P, Ben-David U, Garvie CW, Chen Y, Rees MG, Lyons NJ, McFarland JM, Wong BT, Wang L, Dumont N, O'Hearn PJ, Stefan E, Doench JG, Harrington CN, Greulich H,

- Meyerson M, Vazquez F, Subramanian A, Roth JA, Bittker JA, Boehm JS, Mader CC, Tsherniak A and Golub TR. (2020) Discovering the anticancer potential of non-oncology drugs by systematic viability profiling. *Nat Cancer* 1(2): 235-248.
- Dey P and Chaudhuri TK. (2016) Immunomodulatory activity of *Nerium indicum* through inhibition of nitric oxide and cyclooxygenase activity and modulation of TH 1/TH 2 cytokine balance in murine splenic lymphocytes. *Cytotechnol.* 68(4): 749-761.
- Erdman Jr JW, Balentine D, Arab L, Beecher G, Dwyer JT, Folts J, Harnly J, Hollman P, Keen CL, Mazza G, Messina M, Scalbert A, Vita J, Williamson G and Burrowes J. (2007) Flavonoids and heart health: Proceedings of the ILSI North America flavonoids workshop, May 31–June 1, 2005, Washington, DC. *J Nutr.* 137(3): 718S-737S.
- Giday, M Asfaw Z, Elmqvist T and Woldu Z. (2003) An ethnobotanical study of medicinal plants used by the Zay people in Ethiopia. *J Ethnopharmacol.* 85(1): 43-52.
- Hajhashemi V, Vaseghi G, Pourfarzam M and Abdollahi A. (2010) Are antioxidants helpful for disease prevention?. *Res Pharmaceut Sci.* 5(1): 1.
- Heffner JE and Repine JE. (1989) Pulmonary strategies of antioxidant defense 1. *Am Rev Respir Dis.* 140: 531-554.
- Heneman K and Zidenberg-Cherr S. (2008) *Phytochemicals*. University of California, Publication 8313. <http://anrcatalog.ucdavis.edu/>.
- Jasim H, Hussein AO, Hameed IH and Kareem MA. (2015) Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *J Pharmacog Phytother.* 7(4): 56-72.
- Joshi B, Sah GP, Basnet BB, Bhatt MR, Sharma D, Subedi K, Pandey J and Malla R. (2011) Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem). *J Microbiol Antimicrob.* 3(1): 1-7.
- Kamiloglu S, Sari G, Ozdal T and Capanoglu E. (2020) Guidelines for cell viability assays. *Food Front.* 1(3): 332-349.
- Kaur C and Kapoor HC. (2001) Antioxidants in fruits and vegetables—the millennium's health. *Int J Food Sci Technol.* 36(7): 703-725.
- Kehrer JP. (1993) Free radicals as mediators of tissue injury and disease. *Crit Rev Toxicol.* 23(1): 21-48.
- Kris-Etherton PM, Hecker KD, Bonanome A, Coval SM, Binkoski AE, Hilpert KF, Griel AE and Etherton TD. (2002) Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *Am J Med.* 113(9B): 71S-88S.
- Lu Z, Xiao J, Wang Y and Meng M. (2015) In situ synthesis of silver nanoparticles uniformly distributed on polydopamine-coated silk fibers for antibacterial application. *J Colloid Interf Sci.* 452: 8-14.
- Madhuri S and Pandey G. (2009) Some anticancer medicinal plants of foreign origin. *Curr Sci.* 96(9):779-783.
- McGaw LJ, Elgorashi EE and Eloff JN. (2014) Cytotoxicity of African medicinal plants against normal animal and human cells. In: *Toxicological Survey of African Medicinal Plants*, Elsevier, pp. 181-233.
- Molyneux RJ, Lee ST, Gardner DR, Panter KE and James LF. (2007) Phytochemicals: the good, the bad and the ugly?. *Phytochem.* 68(22–24): 2973-2785.
- Pagliero RJ, D'Astolfo DS, Lelieveld D, Pratiwi RD, Aits S, Jaattela M, Martin NI, Klumperman J and Egan DA. (2016) Discovery of small molecules that induce lysosomal cell death in cancer cell lines using an image-based screening platform. *Assay Drug Dev Technol.* 14(8): 489-510.
- Prasad S. (1949) A brief historical survey of Indian drugs of vegetable origin. *J Am Pharmaceut Assoc.* 38(6): 340-342.
- Quent VM, Loessner D, Friis T, Reichert JC and Huttmacher DW. (2010) Discrepancies between metabolic activity and DNA content as tool to assess cell proliferation in cancer research. *J Cellular Molec Med.* 14(4): 1003-1013.
- Rajasekar K, Muthukumaravel K, Ashok K and Babu M. (2020a) Phytosynthesis of copper oxide nanoparticles from *Opuntia ficus* and its antibreast cancer activity against MCF-7 cell line (invasive ductal carcinoma). *Malaya J Matematik* S(2): 4414-4419.
- Rajasekar K, Muthukumaravel K, Ashok K and Babu M. (2020b) Isolation and primary cell culture of mouse liver cells. *Malaya J Matematik*, S(2): 4430-4432.
- Rietjens IM, Boersma MG, de Haan L, Spenkelink B, Awad HM, Cnubben NH, van Zanden JJ, Woude Hv, Alink GM and Koeman JH. (2002) The pro-oxidant chemistry of the natural antioxidants vitamin C, vitamin E, carotenoids and flavonoids. *Environ Toxicol Pharmacol.* 11(3-4): 321-333.
- Sivagnanam SK, Rao RK, Mudiganti DU and Jeelani PG. (2012) Preliminary phytochemical analysis of

- Artemisia amygdalina*, *Nerium odorum* and *Strychnos potatorum*. J Pharm Res. 5(7): 3734-3739.
- Sumit B and Geetika A. (2012) Therapeutic benefits of holy basil (Tulsi) in general and oral medicine: a review. Int J Res Ayurveda Pharm. 3(6): 761-764.
- Suresh S and Sisodia SS. (2018) Phytochemical and pharmacological aspects of *Cucurbita moschata* and *Moringa oleifera*. Pharmaceut Biosci J. 6(6): 45-53.
- Tapiero H, Townsend DM and Tew KD. (2004) The role of carotenoids in the prevention of human pathologies. Biomed Pharmacother. 58(2): 100-110.
- Valavanidis A, Vlachogianni T, Fiotakis K and Loridas S. (2013) Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. Int J Environ Res Public Hlth. 10(9): 3886-3907.
- Xie X, Van Deusen AL, Vitko I, Babu DA, Davies LA, Huynh N, Cheng H, Yang N, Barrett PQ and Perez-Reyes E. (2007) Validation of high throughput screening assays against three subtypes of Cav3 T-type channels using molecular and pharmacologic approaches. Assay Drug Dev Technol 5(2): 191-204.