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Research Article

GC-MS Profiling and Evaluation of Antioxidant and *invitro* Anticancer Properties of *Alstonia venenata* R.Br. Leaves and Root

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Abstract: The study aimed to evaluate the efficacy of methanolic extracts of *Alstonia venenata* leaves and root for *in vitro* antioxidants and anticancer properties. The exploration of natural products by scientists is a highlighted subject today. Plants synthesize various chemical compounds, which are called secondary metabolites. Antioxidants inhibit oxidation stress and act as protective agents for multiple diseases, including breast cancer. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) have been related in the pathogenesis of many human diseases including cancer. Breast cancer is one of the most destructive types of cancer among women throughout the world.GC-MS analysis was conducted to profile the potential phytocompounds responsible for anticancer activity. Generally, developing new drugs starts with identifying bioactive compounds from natural resources through various analytical techniques.Leaf and root samples were subjected to cold extraction using methanol for plant extraction. Gas chromatography-mass spectrometry was used to identify the phytocompounds present in the extracts. DPPH, ABTS, FRAP, H_2O_2, NO·, ·HO, lipid peroxidation, metal chelating, $O_2^-$and reducing power were determined. Furthermore, MTT assay was performed to assess potential anticancer activity using MCF-7 cell lines.GC-MS analyses of leaf and root methanolic extracts revealed the presence(Methyl-α–d-glucopyranoside,Quinic acid)two and(2-Methylinosine, Methotrexate, 3-O-Methyl-D-Glucose, 2-O-Methyl-D-Xylose, D-Mannoheptulose, Hydroxyurea, Methyl-α–d-Glucopyranoside seven anti-cancerous compounds, respectively. Root extracts than leaf extract showed more antioxidant activities. Higher concentrations of leaf and root methanolic extracts caused a great reduction in the viability of MCF-7 cell lines. Cytotoxicity was found to be relatively higher in root methanolic extract (91.53%) than leaf methanolic extract (71.17%). The present work provides the first evidence for the presence of various anticancer phytocompounds in leaf and root extracts of *A.venenata*

Keywords: *Alstonia venenata,* GC-MS, Antioxidants, Anticancer and MCF-7 cell line

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1. INTRODUCTION

Plant products are known to be important sources of therapeutic agents because they are natural, eco-friendly, and devoid of side effects[1]. Plants with various medicinal properties are nature's gift for human beings because they help to make a disease-free healthy life. They are not linked with their primary metabolism. Instead they serve a variety of ecological functions, including plant's survival during any stress[2]The oxygen molecule produces ROS and RNS due to some exogenous factors such as smoking, air pollutants, radiation, ozone, and industrial chemicals and endogenous metabolic process in human body[3] Enormous formation of ROS and RNS can lead to oxidative stress and begin to display detrimental effects on various cellular structures like proteins, lipids and nucleic acids[4] . Inorder to combat these oxidative stress-related problems, various synthetic antioxidants such as propyl gallate, butylated hydroxyanisole, tert-butylhydroquinone and butylated hydroxytoluene have been vastly used. Yet, the use of such compounds is limited due to their side effects on human well-being. Therefore, attempts have been commenced to explore natural agents as substitutes for artificial antioxidants[5,6]. These compounds have the capability to reduce free radicals, chelate catalytic metals and scavenge oxygen molecules[5,7]. Cancer is a major life-frightening public health issue worldwide, and the unrestricted proliferation of cells[8] denotes it. If cancer is not handled in the primary stages it may lead to cancer metastasis and death. In 2020, there were 2.3 million women detected with breast cancer and 685000 deaths worldwide[9]. In addition, some environmental aspects are also contributing in the development of breast cancer[10]. Therefore, there is imperative to identify naturally occurring anti-carcinogens that would inhibit, slow, or reverse the cancer initiation and development[11,12].The genus *Alstonia* (Apocynaceae) finds a prominent place in various Indian systems of medicines. Several ethnic communities in India have used different species of *Alstonia* to treat various human ailments[13-16].The genus *Alstonia* comprises 43 species, of which *A.scholaris, A.boonei*, *A.congensis*, and *A.macrophylla*are proved useful in various diseases. These species are tropical plants growing in various parts of Africa and South Asia and are highly rich in alkaloids, steroids, and phenolic compounds[17]*Alstonia venenata*R.Br. a medium-sized shrub which is endemic to Eastern and Southern India[18]. Plant-derived various secondary metabolites are being used in cancer treatment. Many studies have shown the involvement of natural plant extracts or plant-derived potential phytocompounds in the formulation of new anticancer drugs with less side effects[19]. Gas chromatography-mass spectrophotometry is one of the reliable methods for screening of various phytocompounds comprising alcohols, alkaloids, nitro compounds, long chain hydrocarbon, organic acids, steroids, esters, and amino acids[20] The present investigation is aimed to evaluate the GC MS, antioxidant and anticancer properties of *A. venenata* leaf and root methanolic extracts. So far, there is no scientific validation on *in vitro* anticancer, antioxidant properties and GC-MS profiling using leaf and root methanolic extracts of *A.venenata*. To fill this research gap, the present research was undertaken.

2. MATERIALS AND METHODS

2.1 *Chemicals*

All the reagents and fine chemicals used in this study were purchased from Sigma-Aldrich, India. Analytical grade of methanolic solvent and other chemicals were procured from Merck, India.

2.2 Collection, Authentication, and Extraction of Plant Materials

Fresh leaf and root of *A. venenata* were collected from Coonoor, Tamil Nadu, India. The plant material was authenticated from Botanical Survey of India, Coimbatore (voucher specimen No: BSI/SRC/5/23/2019/Tech, 3442). Plant samples were thoroughly washed with water and cut into small pieces and shade dried for two weeks. The dried samples were powdered using a mixer grinder and stored at 4°C. The powdered samples were cold extracted using methanol in the ratio of 1:10 (w/v) using an orbital shaker with 180 rpm for 24h at room temperature. The filtrate was concentrated through evaporation at room temperature and dissolved in methanol to get the working solution of 10 mg/ 10 mL (w/v).

2.3 GC-MS Profiling

GC–MS analyses of leaf and root extracts were carried out using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. USA) furnished with a fused silica column, filled with Elite-5MS capillary column (30 m in length × 250 μm in diameter × 0.25 μm in thickness). Helium as carrier gas at a constant flow of 1mL/min was used to separate the components of the extracts. An electron ionization energy protocol was applied with high ionization energy of 70 eV with 0.2 s of scan time and fragments ranging from 40 to 600 m/z for GC–MS spectral identification. The injector column was kept at 260 °C and one μl of sample was injected using this injector into the column. The oven temperature was increased from 60°C to 300°C between 0 and 6 min at the rate of 10°C. The various phytochemicals found in the leaf and root samples were identified through the comparison of their retention time, peak area, peak height ,and mass spectral configurations and also with the database of authentic phytocompounds stored in the National Institute of Standards and Technology (NIST) library and formerly published literature data.

2.4 *In Vitro Antioxidant Activities*

2.4.1 DPPH Radical Scavenging Activity

The DPPH radical scavenging activity of leaf and root methanolic extracts of *A. venenata* was determined according to the method[21]. IC_{50} values of the extract i.e., concentration of extract required to decrease the initial concentration of DPPH by 50% was calculated.

2.4.2 Nitric Oxide Scavenging Ability ($NO^{\cdot}$)

NO. radicals were formed using sodium nitroprusside and quantified by the Greiss reagent at 546 nm using rutin as a standard. All the reactions were carried out in triplicates ,and their percentage inhibition was calculated[22].

2.4.3 Superoxide Radical Scavenging Activity (O_2^{-})

The assay is based on the capability of plant extracts to inhibit formazan formation by scavenging the superoxide radicals generated in riboflavin–light–NBT system[23]The decrease in the extent of NBT is correlated with the superoxide radical scavenging activity of the plant extract.

The percentage of superoxide radical scavenging was calculated using the following equation:

$$(\%) = [A1 - A2)/A1X100]$$

where A1 is the control absorbance and A2 is the sample absorbance.

2.4.4 Hydroxyl Radical Scavenging Activity (OH)

For determination of OH · radical scavenging activity of plant extracts, one mL of the reaction mixture containing 100 µL of 2.8 mM 2-deoxyribose (dissolved in 10 mM phosphate buffer pH 7.4), 100 µl plant extract, 200 µl of 200 µM $FeCl_3$ and 1.04 µM EDTA (1:1 v/v), 100 µl of H_2O_2 (1.0 mM) and 100 µl of ascorbic acid (1.0 mM) was taken. After one hour of incubation at 37ºC, the intensity of the colour produced was measured at 412 nm against reagent blank. The hydroxyl radical scavenging activity of the extracts was calculated as % of antioxidant activity[24].

2.4.5 Lipid Peroxidation Inhibitory Assay

A thiobarbituric acid reactive species (TBARS) assay was used to measure lipid peroxidation inhibition[25].The degree of lipid peroxidation was assayed by calculating the TBARS content in the sample. For this assay, egg yolk homogenate was taken as lipid source, and the Fenton reagent generated free radicals. One mL reaction mixture containing 0.5 mL egg yolk homogenate (10% in distilled water, v/v) and 0.1 mL of extract mixed with 0.05 mL $FeSO_4$ (0.07 M) and incubated for 30 min to induce lipid peroxidation. Free radicals rupture the lipid bilayer and form malonaldehyde (MDA) as a secondary product. Two molecules of thiobarbituric acid react with one molecule of MDA and give a pink-colored product (TBARS). Then 1.5 mL of acetic acid and 1.5 mL of TBA in SDS was added sequentially. The resulting mixture was vortexed and heated at 95ºC for one hour. After cooling, 5 mL of butanol was poured and the mixture was centrifuged at 3000 rpm for 10 min. After removing the supernatant, the intensity of the pink coloured composite was measured using a spectrophotometer at 532 nm. A control experiment was performed in the presence of distilled water without the extract and the percentage of inhibition of lipid peroxidation was determined.

2.4.6 Hydrogen Peroxide Scavenging Activity (H_2O_2)

To determine H_2O_2 scavenging activity of the plant extracts, 4 mM hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). Out of this, 600 µl was added to the plant sample and finally, the volume was made into 4 mL with phosphate buffer. Samples were incubated for 10 minutes, absorbance was taken at 230 nm using rutin as a control, and percentage inhibition was calculated[26].

2.4.7 ABTS Radical Scavenging Activity

Free radical scavenging nature of plant extracts was determined by ABTS radical cation decolorization assay. The percentage of ABTS+ scavenging activity of the crude extracts and standard rutin was calculated using the formula. Results were expressed as µM Trolox equivalent capacity per gram of sample extract [27]

2.4.8 Ferric Reducing Antioxidant Power (Frap)

The FRAP reagent was freshly prepared and warmed at 37°C. A 900 µl FRAP reagent was mixed with 90 µl water and 30 µl of the plant sample. The reaction mixture was incubated at 37°C for 10 min ,and the absorbance was taken at 593 nm. FRAP was expressed as mmol Fe (II)/g extract[28].

2.4.9 Metal Chelating Activity

The chelating activity of ferrous ions by plant extracts was determined by taking absorbance at 560 nm and the results were expressed as mg EDTA equivalent/g extract[29].

2.4.10 Reducing Power Assay

Plant extracts with different concentrations were added with 0.2 mL of 0.2 M phosphate buffer (pH 6.6) and 0.2 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min and then 0.25 mL of trichloroacetic acid was added. After centrifugation, supernatant was collected and added with 0.1 mL of 0.1% $FeCl_3$. Absorbance was recorded at 700 nm and results were expressed as mg RU Eq/mL.[30]

2.5 In Vitro Anticancer Activity

2.5.1 Cell Culture

MCF-7 (Human breast cancer cells) cell line was procured from NCCS, Pune and was cultured in liquid medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 µg/mL penicillin and 100 µg/mL streptomycin and maintained under an atmosphere of 5% CO_2 at 37ºC.

2.5.2 MTT Assay

Leaf and root methanolic extracts of *A. venenata* was subjected to *in vitro* cytotoxicity assay using MCF-7 cell lines by MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay[31,32]. Briefly, the cultured MCF-7 cells were harvested by trypsinization and pooled in a 15 mL tube. Then, the cells were plated at a density of 1×10^5 cells/mL cells/well (200 µl) into the 96-well tissue culture plate containing 10 % FBS and 1% antibiotic solution (DMEM) for 24-48 h at 37°C. The wells were washed with sterile PBS and treated with five different concentrations (100, 200, 300, 400 and 500 µg/mL) of the plant extracts in a serum free DMEM medium. Each sample was replicated three times and the cells were incubated at 37°C in a humidified 5% CO_2 incubator for 24 h. After the incubation time, MTT (20 µl of 5 mg/mL) was added into each well and the cells were incubated for another 2-4 h until purple precipitates were clearly visible under an inverted microscope. Finally, the medium together with MTT (220 µl) were aspirated off the wells and washed with 1X PBS (200 µl). DMSO (100 µl) was added to dissolve formazan crystals and the plate was shaken for 5 min. The absorbance for each well was measured at 570 nm using a microplate reader (Thermo Fisher Scientific, USA) and the percentage cell viability and IC_{50} value were calculated using Graph Pad Prism 8.0 software (USA).

3. STATISTICAL ANALYSIS

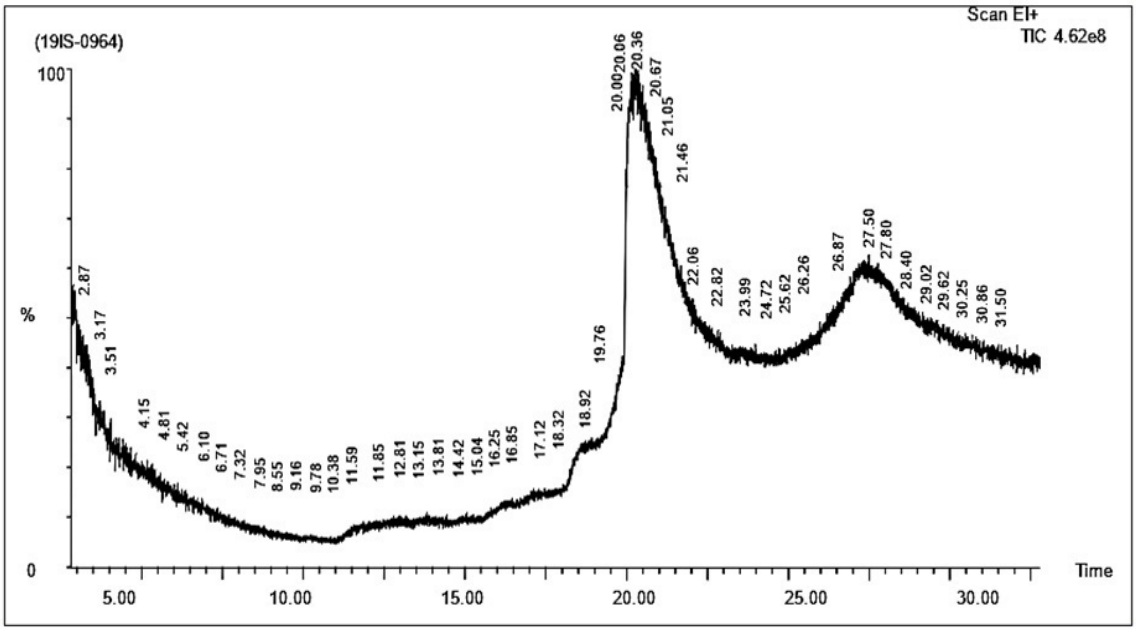
All the data were subjected to one-way ANOVA followed by DMART test for post-hoc analysis. The statistical level of significance was fixed at $p<0.05$. SPSS software, version 13.0

(SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Data were represented as the means±SD. MTT Assay was calculated using Graph Pad Prism 8.0 software (USA).

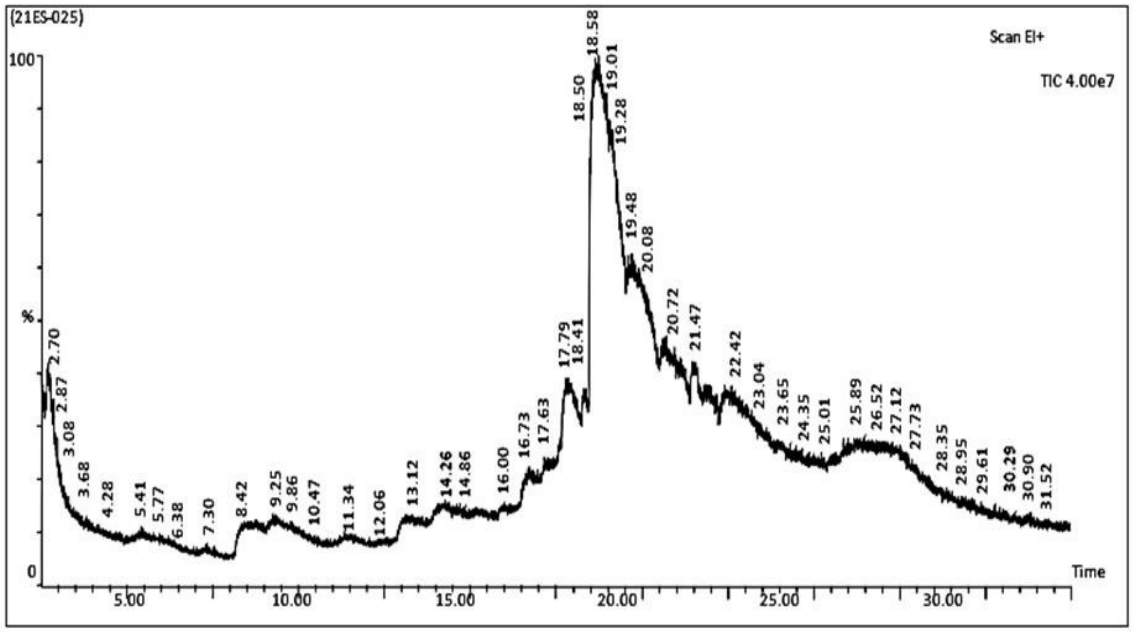
4. RESULTS AND DISCUSSION

4.1 GC-MS Analysis

In the present study, methanolic extracts of *A.venenata* leaf and root were subjected to GC-MS analysis in order to identify the phytoconstituents responsible for anticancer activity. GC-MS is one of the most commonly used analytical techniques for separating and identifying various phytocompounds[33]. Totally 74 phytocompounds were identified in both the extracts, of which nine compounds were found to have anticancer properties. Methanolic leaf (LME) and root extracts (RME) of GC-MS profiles are shown in Figure 1 (a) and Figure 1(b), respectively. Leaf and root samples of GC-MS Chromatograms recorded two and seven anticancer phyto-components, respectively (Table1 and Table 2). These phytocompounds were identified by comparing their retention time and peak area (%) to known compounds quoted by NIST library. Other than two anticancer phytocompounds, such as, methyl-alpha–d-glucopyranoside and quinic acid, leaf methanolic extract was found to possess 12 various phytoconstituents. Root methanolic extract had 53 phyto-components in addition to seven anticancer constituents. Methyl-α-d-glucopyranoside from *Tulbaghia violacea* aqueous extract induces apoptosis*i nvitro* cancer cells such as MCF-7 and HeLa cell lines[34] Quinic acid was found to be a potent drug for prostate cancer through *insilico* analysis due to its higher drug score i.e. 0.48 and drug-likeness feature, i.e., 0.51 with lesser brain permeation and reduced toxicity nature[35]. Nucleosides analogs are important components of treatment regimens for various cancer types with circumventing development of resistance[36]Methylinosineis one of the nucleosides and clinical tumour burden was not reflected in patients with breast carcinoma treated with methylinosine[37] Similarly, clinical outcome was found better for this chemical than other two nucleosides used, i.e,pseudouridine and 2, methyl adenosine to treat 31 patients with cancer of the female urinary organs [38]Methotrexate has been used in the treatment of various types of cancer such as breast, brain, head, neck, lung and also to cure hepatoma, lymphomas, esophagogastric carcinomas, osteosarcoma, and gastric cancer[39,40] In addition, it is taken in combination with other drugs to treat various neoplasms[41-43]Wozniak et al. (2021)designed, synthesized and evaluated an innovative glucose-methotrexate conjugate and have shown that it has broad spectrum antitumor activity for various cell lines than unconjugated drug[44]. The protective nature of 3-O-methyl-D-glucose against streptozotocin toxicity andleukamia cancer was reported[45]. Recently, this chemical has been utilized as glucose analogue in Chemical Exchange Saturation Transfer (CEST) MRI scanning experiments for detection of breast cancer[46,47] 2-O-methyl-xylose is a rhamnogalacturonan-II-type of polysaccharide and it has anti-tumor and anti-metastatic activities with the activation of macrophages and natural killer cells[48].D-monoheptulose is a non-metabolizable glucose analog which inhibits hexokinase enzyme which in turn block glucose metabolism (glycolysis) in cancer cells. Since cancer cells depend on glycolysis for ATP and other products for their growth and proliferation, therefore, inhibition of glucose metabolism might be of therapeutic value in anticancer treatment. Recent studies with D-monoheptulose alone and in combination with Newcastle Disease Virus could inhibit the growth and multiplication of breast cancer cells by inhibiting glucose metabolism[49] This effect was manifested by apoptotic cell death, downregulation of hexokinase enzyme and reduced level of glycolysis products. Hydroxyurea is a valuable drug for treating some types of cancer such as myelocyticleukemia[50,-52] ovary and cervical carcinoma[53,54], melanoma and meningioma[55,56]and carcinoma of head and neck[57-60]Hydroxyurea inhibits the activity of iron-dependent enzyme i.eriboculeotide reductase enzyme which is responsible for the conversion of ribonucleotides into deoxyribonucleotides[61]. The broad spectrum action of this drug is due to its passive diffusion behaviour into brain and cerebrospinal fluid[62,63]



(a)



(b)

Inst() ACQUISITION PARAMETERS

Oven: Initial temp 60° C for 2.80min, ramp10°c/minto300°C, hold6min, InjAauto=260°C, Volume=1µl, split=10:1, Carriergas=He, Solvent =2.00min, Transfer Temp=230°C, Source Temp=230°C, Scan:50 to 600Da, Column 30.0m ×250 µm

Fig 1: GC-MS chromatogram of leaf (a) and root (b) methanolic extract of *A. venenata*

Table 1: Anticancerphytocompounds identified in the methanolic leaf extract of *A.venenata*

No.	Compound Name	Molecular formula	Molecular weight	Retention Time	Area%
1	Methyl-α-d-glucopyranoside	$C_7H_{14}O_6$	194.18	20.281	84.900
2	Quinic acid	$C_7H_{12}O_6$	192.11	27.504	15.100

Table 2: Anticancerphytocompounds identified in the methanolic root extract of *A.venenata*

No.	Compound Name	Molecular formula	Molecular weight	Retention Time	Area%
1	2-Methylinosine	$C_{11}H_{14}O_5N_4$	282.25	17.880	3.467
2	Methotrexate	$C_{20}H_{22}O_5N_8$	454.40	18.755	62.269
3	3-O-Methyl-D-Glucose	$C_7H_{14}O_6$	194.18	20.671	9.056
4	2-O-Methyl-D-Xylose	$C_6H_{12}O_5$	164.16	21.576	6.289
5	D-Mannoheptulose	$C_7H_{14}O_7$	210.18	22.632	9.141
6	Hydroxyurea	$CH_4O_2N_2$	76.05	26.213	3.284
7	Methyl- α -d-Glucopyranoside	$C_7H_{14}O_6$	194.18	26.518	6.495

The chief phyto constituents identified in the methanolic leaf and root extracts are given in Table 1&2 respectively.GC–MS chromatogram of methanolic leaf and root extracts of *A. venenata recorded* a total of 9 peaks corresponding to the bioactive compounds that were recognized by relating their peak retention time, peak area (%) and mass spectral fragmentation patterns to that of the known compounds described by the National Institute of Standards and Technology (NIST) library.

4.2 In Vitro Antioxidant Activities

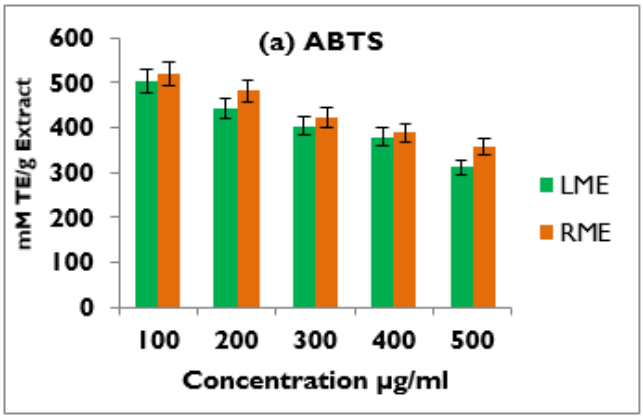
Reactive Oxygen Species or free radicals are involved in the pathogenesis of various diseases because of the oxidization of biomolecules followed by the emergence of oxidative stress[64] Natural antioxidants such as polyphenols, flavonoids and phenolic compounds present in plants are responsible for inhibiting the harmful effects of oxidative stress[65]There are numerous scientific data on the importance of natural antioxidants for human health[66]and a relationship between the intake of plant-rich antioxidants and the reduced risk of many cancer diseases[67-71]. *In vitro* DPPH, $NO^{\cdot}$, $O_2^{\bullet-}$, $OH^{\cdot}$, H_2O_2, lipid peroxidation scavenging activities of LME and RME of *A. venenata* is given in the Table 3. Among various concentrations used, 100 µg/mL showed low IC_{50} value than other concentrations for all the radical scavenging assays. However, RME extract showed still lower IC_{50} than LME (DPPH 7.5±1.3 µg/ascorbic acid/mL; $NO^{\cdot}$ - 5.8±1.8 µg/rutin/mL; $O2^{\bullet-}$ - 6.2±1.7µg/rutin/mL; $OH^{\cdot}$ -6.7±1.0 µg/rutin/mL; H_2O_2- 4.6±1.3µg/rutin/mL and lipid peroxidation- 5.2±1.0µg/rutin/mL). DPPH free radical scavenging is an accepted mechanism for understanding the antioxidant activity of plant extracts[72].DPPH assay is primarily based at the potential of the stable loose radical 2,2-diphenyl-1-picrylhydrazyl to react with hydrogen donors[73]NO scavenging assay has been used to evaluate the antioxidant potential of the plant extracts which is generated from amino acid L-arginine by the enzymes of vascular endothelial cells, certain neuronal cells and phagocytes[74]. Low concentrations of NO are a disseminative free radical that plays

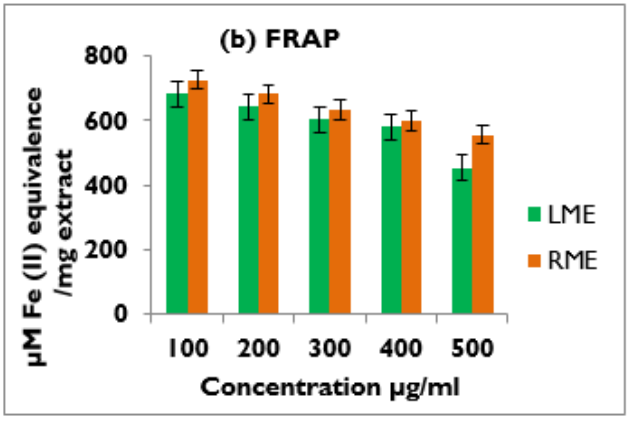
miscellaneous roles as an effectors molecule in various organic systems which include neuronal messenger, vasodilatation, antimicrobial and antitumor activities[75] However, enormous production leads to various oxidative relative diseases[76], which is directly scavenged by flavonoids[77]All the unfastened radicals which include O2•- are produced constantly during metabolism. The excessive level of superoxide radical is harmful to biological material which causes numerous diseases[78] OH · radical is the most reactive oxygen species. It induces numerous damages in diverse biomolecules[79] Hydroxyl radical is produced through the Fenton reaction in the presence of decreased transition metals which include as Fe2+ and H_2O_2[80].Mitigating of hydroxyl radical is a vital due to its excessive reactivity with a huge variety of biomolecules[81] Lipid peroxidation has been related to cell injury in biomembranes[82] and it leads to DNA injury, inflammation and succeeding cell death[83] H_2O_2generated in an organic system by numerous oxidizing enzymes[84] and its accumulation is liable for oxidative stress and inflammation reactions[85] . Previous studies with increased free radical scavenging activities of methanolic extract for DPPH-*Plectranthus stockii*[86] *Moringo concanensis*[87] *Sphagneticola trilobata*[88] and *Dioscorea bulbifera*[89] NO-*Spondias pinnata*[90]*Aeglemarmelos*[91]and *Rhododendron arboretum*[92]$O_2^{\bullet-}$, OH ·, H_2O_2-*Asparagus racemosus*[93]and lipid peroxidation-*Tabebuia pallida*[72]. ABTS radical scavenging activity, ferric reducing antioxidant power, metal chelating activity and reducing power of LME and RME are presented in Figure2 (a-d). Based on the results obtained, the scavenging ability of all the concentrations of both samples could be ranked as follows: 100>200>300>400>500. Of the two extracts, RME (100 µg/mL)showed the highest activity for all the free radicals than LME i.e $p<0.05$ for ABTS- 518.7±1.5mM TEq/g; FRAP-725.5±5.1µmFe(II) Eq/mg; metal chelating activity-783.3±2.8 Mm EDTA Eq/g Extract and reducing power-689.8±1.3 mg RU Eq/g Extract. The relatively stable ABTS radical is recommended for use in the determination of antioxidant activity of plant extracts [94]. During FRAP assay, Fe^{3+} has been transformed into Fe^{2+} in plant methanolic extract, a measure of antioxidant power[95]. Excess free irons an implicated in the induction and formation of unfastened free radicals in the biological systems. Phytocompounds with iron chelating capacity can act as effective antioxidants [96] Ferrozine produces a violet complex with Fe^{2+} and in the presence of a chelating agent or plant extract the violet color of the complex is decreased and the reduction of colour is a direct estimation of chelating metal activity[90] The reducing power assay is often used to determine the capability of an antioxidant to donate an electron[97]The antioxidant compounds present in the plant extract convert the oxidation form of iron (Fe^{+3}) of ferric chloride to ferrous (Fe^{+2}) during reducing power assay [98]

Table 3: *In vitro* DPPH, $NO^{\cdot}$, $O_2^{\bullet-}$, OH ; H_2O_2, lipid peroxidation scavenging activities of LME and RME

Methanolic Extract	DPPH (IC_{50}) Ascorbic acid	$NO^{\cdot}$ (IC_{50}) Rutin	$O_2^{\bullet-}$ (IC_{50}) Rutin	OH · (IC_{50}) Rutin	H_2O_2 (IC_{50}) Rutin	Lipid peroxidation Rutin
Con.(µg/ml)			LME			
100	10.5±1.7^a	10.2±1.8^a	9.8±1.4^a	12.2±1.4^a	17.3±1.8^a	9.5±1.0^a
200	19.3±1.9^b	18.3±1.9^b	19.6±1.0^b	16.3±1.2^b	23.3±1.2^b	15.3±1.2^b
300	30.5±1.5^c	28.5±1.5^c	25.2±1.4^c	23.5±1.5^c	26.5±1.5^c	20.5±1.5^c
400	36.5±1.2^d	35.5±1.2^d	31.2±1.2^d	38.5±1.2^d	28.5±1.2^d	25.5±1.2^d
500	40.0±1.3^e	46.0±1.3^e	38.2±1.0^e	43.0±1.3^e	32.0±1.3^e	36.0±1.3^e
Con.(µg/ml)			RME			
100	7.5±1.3^a	5.8±1.8^a	6.2±1.7^a	6.7±1.0^a	4.6±1.3^a	5.2±1.0^a
200	12.3±1.2^b	16.3±1.2^b	18.3±1.2^b	15.9±1.2^b	16.2±1.2^b	13.2±1.2^b
300	22.3±1.5^c	28.3±1.5^c	21.3±1.5^c	20.0±1.5^c	23.0±1.5^c	18.0±1.5^c
400	28.6±1.8^d	32.6±1.8^d	25.6±1.8^d	24.6±1.8^d	25.6±1.8^d	23.7±1.8^d
500	34.5±1.9^e	38.5±1.9^e	32.5±1.9^e	30.8±1.9^e	35.8±1.9^e	25.0±1.9^e

DPPH- 2, 2-diphenylpicrylhydrazylradical scavenging activity (ascorbic acid equivalence); Nitric oxide (rutin equivalence); $O_2^{\bullet-}$- super oxide (rutin equivalence); OH ·-Hydroxyl radical (rutin equivalence); H_2O_2- Hydrogen peroxide (rutin equivalence); lipid peroxidation (rutin equivalence);IC_{50} - inhibition concentration for 50%; LME-Leaf methanolic extract; RME- Root methanolic extract. Values are statistically significant at $p< 0.05$ where a>b>c>d>e. Where all values are represented as mean ±SD (n=3).





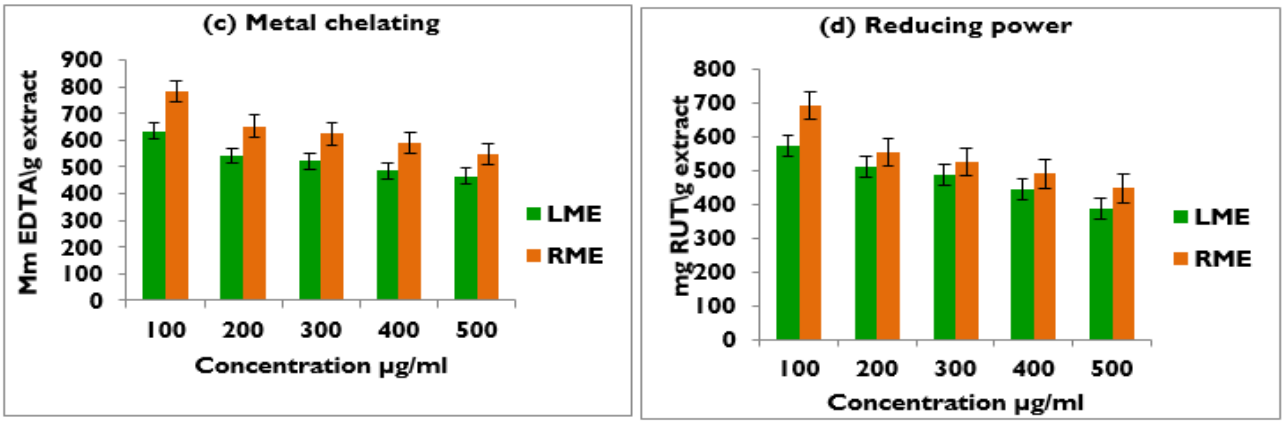


Fig 2:(a) ABTS(b) FRAP (c) Metal chelating and(d) Reducing power activities of LME and RME values are represented as mean ± SD (n = 3).

4.3 Anticancer Activity of A.Venenata

Herbal drugs have a vital role in the treatment of cancer[99]The aim of this assay was to evaluate the inhibitory or cytotoxic activity of LME and RME extracts on cancer cell lines proliferation. Cell viability was quantified by MTT assay following 24hr exposure to extracts (100-500µg/mL). The percentage of cell death increased as the concentration of the extract increased.RME showed highest cell death i.e 91.53%, compared to LME extract i.e 71.17%. The cytotoxic activity of LME and RME was measured as IC_{50} value or the amount of extract required to inhibit the 50% cell growth with reference to untreated 100% living cells[100]There was a significant difference in the IC_{50} values between LME and RME(49.06 µg/mL and12.30 µg/mL), respectively, which indicated that RME exerted more potent anticancer activity on MCF-7 cells than LME (Table 4).Figure 3 shows morphological changes such as cytoplasmic condensation, cell shrinkage and production of numerous cell surface swelling at the plasma membrane when 500 µg/ mL used Methanolic extract reveal the highest cytotoxic activity indicate that antioxidants play the most important responsibility in cytotoxicity[101]. In a previous report of the methanol extract of *Jacaranda obtusifolia* H. B. K. ssp. *Rhombifolia* Gentryin twig extracts revealed anticancer activity in opposition to the NCI-H187 (small cell lung cancer) cell line with an IC50 of 23.2 mg/mL[102].Pearson correlation regression analysis was also carried out between all the antioxidant and anticancer activities. A positive linear correlation (0.910-0.987 for LME; 0.915-0.990 for RME) was noted between free radical scavenging and anti-proliferative activities. However, the highest significant correlation ($p<0.01$) was obtained for RME than LME (Table 5). These correlations powerfully imply the concern of diverse compounds in antioxidant activities. To the best of our information, this study represents the first instance in which the anticancer potential of A.venenata Methanolic leaf and root extract against MCF-7 cell lines has been demonstrated.

Table 4: Cytotoxicity of LME and RME of *A.venenata* on MCF-7 cell line

NO.	Concentration (µg/ml)	Cell Death on MCF-7 cell line (%)	IC_{50}value (µg/ml)
		LME	
1.	100	48.18	49.06 µg/ml
2.	200	54.37	
3.	300	55.99	
4.	400	67.61	
5.	500	71.17	
		RME	
1.	100	54.29	12.30 µg/ml
2.	200	62.52	
3.	300	69.22	
4.	400	79.23	
5.	500	91.53	

Concentrations of studied substances that caused a 50% decrease in cell viability with reference to untreated controls (IC_{50}) of extracts on MCF-7 cell line; LME-Leaf Methanolic extract; RME-Root Methanolic extract

Table 5: Correlation between free radical scavenging activities and anticancer activity of LME and RME

		Correlation (R^2)									
Cell line	Sample	DPPH	$NO^{\cdot}$	$O_2^{\bullet-}$	$OH^{\cdot}$	H_2O_2	LPO	ABTS	FRAP	Metal chelating	Reducing power assay
MCF-7	LME	0.940*	0.957*	0.966**	0.987**	0.938*	0.959*	0.945*	0.910*	0.924*	0.973**
	RME	0.981**	0.955*	0.962**	0.980**	0.941*	0.948*	0.976**	0.990**	0.921*	0.915*

**correlation coefficient is significant at the 0.05 level; **correlation coefficients is significant at the 0.01 level*

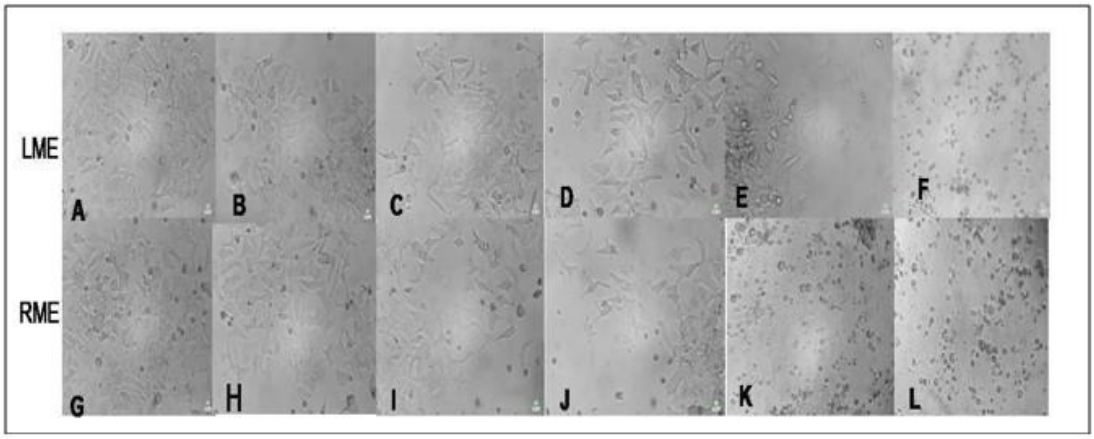


Fig 3: Morphological changes in MCF-7 Cell lines treated with LME and RME

Morphological changes in cells of MCF-7 after treatment with leaf methanolic extract (LME) and root methanolic extract (RME) of *A. venenata* A: (Untreated cells) B-F: *A. venenata*LME, B-100 µg/mL; C-200 µg/mL; D-300 µg/mL; E-400 µg/mL; F-500 µg/mL (Untreated cells) H-L: *A. venenata*RME, H-100 µg/mL; I-200 µg/mL; J-300 µg/mL; K-400 µg/mL; L-500 µg/mL

5. CONCLUSIONS

In the present investigation, our research findings indicate that the root methanolic extract of *A. venenata* possesses satisfactory *in vitro* anticancer activity than leaf methanolic extract. The higher anti-proliferative activity of root extract might be due to more free radical scavenging activities, as evidenced by various *in vitro* antioxidant assays. The prevention of oxidative stress is a valuable property of all medicinal plants when treating emphasis disorders. This study is the first report of leaf and root methanolic extracts of *A.venenata*. In the lab, additional research is being done to clarify the mechanisms of action of cell death in breast cancer and to pinpoint the active ingredients. The pharmaceutical industry can use these bioactive molecules to create lead medications to treat cancer and germs in the present and the future. They are effective in treating a wide range of diseases. Further studies are underway to determine the bioactive principle/s of this extract behind this activity using *in vivo* animal models.

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8. AUTHORS CONTRIBUTION STATEMENT

J. Aarthi designed the whole study, including sample collection, GC MS, Antioxidant assay, *invitro* anticancer activity at Department of Botany, Bharathiar University, and prepared the manuscript. N.Geetha prepared the contribution part of the manuscript. All the authors read and approved the final version of the manuscript.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. George P. Concerns regarding the safety and toxicity of medicinal plants-An overview. J Appl Pharm Sci. 2011;1(6):40-4.
2. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and naturalproducts. Indian J Pharmacol. 2000;32(4):S81-118.
3. Haq SH, Al-Ruwaished G, Al-Mutlaq MA, Naji SA, Al-Mogren M, Al-Rashed S et al. Antioxidant, anticancer activity and phytochemical analysis of green algae, Chaetomorpha collected from the Arabian Gulf. Sci Rep. 2019;9(1):1-7.
4. Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense systems, and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 2013;46:200-6. doi: 10.1016/j.pnpbp.2013.02.015, PMID 23470289.
5. Złotek U, Mikulska S, Nagajek M, Świeca M. The effect of different solvents and number of extraction steps on the polyphenol content and antioxidant capacity of basil leaves OcimumbasilicumL.extracts. Saudi J Biol Sci. 2016;23(5):628-33. doi: 10.1016/j.sjbs.2015.08.002, PMID 27579013.
6. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S et al. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophila aromatica. J Food Drug Anal. 2014;22(3):296-302. doi: 10.1016/j.jfda.2013.11.001, PMID 28911418.
7. Zhao Y, Chen S, Wang Y, Lv C, Wang J, Lu J. Effect of drying processes on prenylflavonoid content and antioxidant activity of EpimediumkoreanumNakai. J Food Drug Anal. 2018;26(2):796-806. doi: 10.1016/j.jfda.2017.05.011, PMID 29567251.

8. Lim GC. Overview of cancer in Malaysia. Jpn J Clin Oncol. 2002;32;Suppl:S37-42. doi: 10.1093/jjco/hye132, PMID 11959876.
9. WHO Breast cancer: March 26. p. 2021Date. Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancerDate [accessed: Dec 20, 2021].
10. Akhouri V, Kumari M, Kumar A. Therapeutic effect of Aegle marmelos fruit extract against DMBA induced breast cancer in rats. Sci Rep. 2020;10(1):18016. doi: 10.1038/s41598-020-72935-2, PMID 33093498.
11. Sahayarayan JJ, Rajan KS, Vidhyavathi R, Nachiappan M, Prabhu D, Alfarraj S et al. In-silico protein-ligand docking studies against the estrogen protein of breast cancer using pharmacophore based virtual screening approaches. Saudi J Biol Sci. 2021;28(1):400-7. doi: 10.1016/j.sjbs.2020.10.023, PMID 33424323.
12. Devasagayam TP, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. Indian J Exp Biol. 2002;40(6):639-55. PMID 12587713.
13. Shankar R, Singh VK, Rawat MS. Medicinal plants from Dibang valley (Arunachal Pradesh): social forestry and afforestation. Bull Med-Ethnogr Bot Res. 1993;14:(3-4).
14. Pant SC, Pandey G. Ethno-botanical studies on medicinal flora in Tharu tribal pockets in Kumaon region in Uttar Pradesh. Bull Med Ethnogr Bot Res. 1995;16(1&2):1-20.
15. Prasad PN, Jebadhas AW, Janaki Ammal EK. Medicinal plants used by the Kanikkars of South India. J Econ Taxon Bot. 1987;11(1):149-55.
16. Sur PR, Sen R, Halder AC, Bandyopadhyay S. Observation on the ethnobotany of Malda-West Dinajpur districts, West Bengal-II. J Econ Taxon Bot:1990.14(2):453-459.
17. Pratyush K, Misra CS, James J, Dev LM, Veettil AK, Thankamani V. Ethnobotanical and pharmacological study of Alstonia (Apocynaceae)-A review. J Pharm Sci Res. 2011;3(8):1394.
18. Kulkarni VA, Seetharam YN, Rao KS. AlstoniaVenenata R. BR.(Apocynaceae): A note on distribution pattern of an endemic species. Nelumbo. 2011;53:211-2.
19. Azam K, Nur M, Rahman M, Biswas S, Ahmed M. Appraisals of Bangladeshi medicinal plants used by folk medicine practitioners in the prevention and management of malignant neoplastic diseases. Int Sch Res Not. 2016;16:1-12.
20. Razack S, Kumar KH, Nallamuthu I, Naika M, Khanum F. Antioxidant, biomolecule oxidation protective activities of Nardostachysjatamansi DC and its phytochemical analysis by RP-HPLC and GC-MS. Antioxidants (Basel). 2015;4(1):185-203. doi: 10.3390/antiox4010185, PMID 26785345.
21. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200. doi: 10.1038/1811199a0.
22. Sreejayan MN, Rao MN. Nitric oxide scavenging by curcuminoids. J Pharm Pharmacol. 1997;49(1):105-7. doi: 10.1111/j.2042-7158.1997.tb06761.x, PMID 9120760.
23. Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal Biochem. 1971;44(1):276-87. doi: 10.1016/0003-2697(71)90370-8, PMID 4943714.
24. Klein SM, Cohen G, Cederbaum AI. Production of formaldehyde during metabolism of dimethyl sulfoxide by hydroxyl radical-generating systems. Biochemistry. 1981;20(21):6006-12. doi: 10.1021/bi00524a013, PMID 6272833.
25. Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. Food Chem. 2000;69(2):167-74. doi: 10.1016/S0308-8146(99)00247-2.
26. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989;10(6):1003-8. doi: 10.1093/carcin/10.6.1003, PMID 2470525.
27. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-7. doi: 10.1016/s0891-5849(98)00315-3, PMID 10381194.
28. Pulido R, Bravo L, Saura-Calixto F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. J Agric Food Chem. 2000;48(8):3396-402. doi: 10.1021/jf9913458, PMID 10956123.
29. Dinis TC, Maderia VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch Biochem Biophys. 1994;315(1):161-9. doi: 10.1006/abbi.1994.1485, PMID 7979394.
30. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. JpnJNutrDiet. 1986;44(6):307-15. doi: 10.5264/eiyogakuzashi.44.307.
31. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods. 1983;65(1-2):55-63. doi: 10.1016/0022-1759(83)90303-4, PMID 6606682.
32. Wilson AP. Cytotoxicity and viability assays. Animal cell culture: A Practical Approach. 2000;1:175-219.
33. Konappa N, Udayashankar AC, Krishnamurthy S, Pradeep CK, Chowdappa S, Jogaiah S. GC–MS analysis of phytoconstituents from Amomumnilgiricum and molecular docking interactions of bioactive serverogeninacetate with target proteins. Sci Rep. 2020;10(1):16438. doi: 10.1038/s41598-020-73442-0, PMID 33009462.
34. Lyantagaye SL. Methyl-α-D-glucopyranoside from Tulbaghia violacea extract induces apoptosis in vitro in cancer cells. Bangladesh J Pharmacol:2013.8(2):93-101.
35. Inbathamizh L, Padmini E. Quinic acid as a potent drug candidate for prostate cancer–a comparative pharmacokinetic approach. Asian J Pharm Clin Res. 2013;6(4):106-12.
36. Zhang J, Visser F, King KM, Baldwin SA, Young JD, Cass CE. The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. Cancer Metastasis Rev. 2007;26(1):85-110. doi: 10.1007/s10555-007-9044-4, PMID 17345146.
37. Tormey DC, Waalkes TP, Gehrke CW. Biological markers in breast carcinoma clinical correlations with pseudouridine, n2, N2-dimethylguanosine and

1-methylinosine. J Surg Oncol. 1980;14(3):267-73. doi: 10.1002/jso.2930140313, PMID 7392649.
38. Koshida K, Harmenberg J, Stendahl U, Wahren B, Borgström E, Helström L et al. Urinary modified nucleosides as tumor markers in cancer of the urinary organs or female genital tract. Urol Res. 1985;13(5):213-8. doi: 10.1007/BF00261578, PMID 4060364.
39. Wong PT, Choi SK. Mechanisms and implications of dual-acting methotrexate in folate-targeted nanotherapeutic delivery. Int J Mol Sci. 2015;16(1):1772-90. doi: 10.3390/ijms16011772, PMID 25590303.
40. Khan ZA, Tripathi R, Mishra B. Methotrexate: a detailed review on drug delivery and clinical aspects. Expert Opin Drug Deliv. 2012;9(2):151-69. doi: 10.1517/17425247.2012.642362, PMID 22251428.
41. Huennekens FM. The methotrexate story: a paradigm for development of cancer chemotherapeutic agents. Adv Enzyme Regul. 1994;34:397-419. doi: 10.1016/0065-2571(94)90025-6, PMID 7942284.
42. Bonadonna G, Valagussa P, Moliterni A, Zambetti M, Brambilla C. Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer the results of 20 years of followup. N Engl J Med. 1995;332(14):901-6. doi: 10.1056/NEJM199504063321401, PMID 7877646.
43. Crews KR, Liu T, Rodriguez-Galindo C, Tan M, Meyer WH, Panetta JC et al. High-dose methotrexate pharmacokinetics and outcome of children and young adults with osteosarcoma. Cancer. 2004;100(8):1724-33. doi: 10.1002/cncr.20152.
44. Woźniak M, Pastuch-Gawołek G, Makuch S, Wiśniewski J, Krenács T, Hamar P et al. In vitro and in vivo Efficacy of a Novel Glucose-Methotrexate Conjugate in Targeted Cancer Treatment. Int J Mol Sci. 2021;22(4):1748. doi: 10.3390/ijms22041748, PMID 33572433.
45. Wick MM, Rossini A, Glynn D. Reduction of streptozotocin toxicity by 3-O-methyl-D-glucose with enhancement of antitumor activity in murine L1210 leukemia. Cancer Res. 1977;37(11):3901-3. PMID 198124.
46. Rivlin M, Navon G. CEST MRI of 3-O-methyl-D-glucose on different breast cancer models. Magn Reson Med. 2018;79(2):1061-9. doi: 10.1002/mrm.26752, PMID 28497566.
47. Anemone A, Consolino L, Conti L, Irrera P, Hsu MY, Villano D et al. Tumour acidosis evaluated in vivo by MRI-CEST pH imaging reveals breast cancer metastatic potential. Br J Cancer. 2021;124(1):207-16. doi: 10.1038/s41416-020-01173-0, PMID 33257841.
48. Park HR, Hwang D, Suh HJ, Yu KW, Kim TY, Shin KS. Antitumor and antimetastatic activities of rhamnogalacturonan-II-type polysaccharide isolated from mature leaves of green tea via activation of macrophages and natural killer cells. Int J Biol Macromol. 2017;99:179-86. doi: 10.1016/j.ijbiomac.2017.02.043, PMID 28223130.
49. Silver RT, Woolf SH, Hehlmann R, Appelbaum FR, Anderson J, Bennett C et al. An evidence-based analysis of the effect of busulfan, hydroxyurea, interferon, and allogeneic bone marrow transplantation in treating the chronic phase of chronic myeloid leukemia: developed for the American Society of Hematology: Presented in part at the Education Session of the American Society of Hematology. FL: Miami Beach; December 5, 1998. Blood, The Journal of the American Society of Hematology.1999 94(5):1517-36.
50. Goldman JM. Optimizing treatment for chronic myeloid leukemia. N Engl J Med. 1997;337(4):270-1. doi: 10.1056/NEJM199707243370410, PMID 9227935.
51. Hehlmann R, Berger U, Pfirrmann M, Hochhaus A, Metzgeroth G, Maywald O et al. Randomized comparison of interferon α and hydroxyurea with hydroxyurea monotherapy in chronic myeloid leukemia (CML-study II): prolongation of survival by the combination of interferon α and hydroxyurea. Leukemia. 2003;17(8):1529-37. doi: 10.1038/sj.leu.2403006, PMID 12886239.
52. Piver MS, Barlow JJ, Vongtama V, Blumenson L. Hydroxyurea: A radiation potentiat-or in carcinoma of the uterine cervix: A randomized double-blind study. Am J Obstet Gynecol;7: 803-808:1983147.
53. Hreshchyshyn MM, Aron BS, Boronow RC, Franklin III EW, Shingleton HM, Blessing JA. Hydroxyurea or placebo combined with radiation to treat stages IIIB and IV cervical cancer confined to the pelvis. Int J Radiat Oncol Biol Phys. 1979;5(3)* Biology* Physics.1979 15(3):317-22. doi: 10.1016/0360-3016(79)91209-4, PMID 110744.
54. Schrell UM, Rittig MG, Koch U, Marschalek R, Anders M. Hydroxyurea for treatment of unresectablemeningiomas. Lancet. 1996;348(9031):888-9. doi: 10.1016/S0140-6736(05)64757-5, PMID 8826822.
55. Schrell UM, Rittig MG, Anders M, Kiesewetter F, Marschalek R, Koch UH et al. Hydroxyurea for treatment of unresectable and recurrent meningiomas. I. Inhibition of primary human meningioma cells in culture and in meningioma transplants by induction of the apoptotic pathway. Neurosurg Focus. 1997;2(4):E10.
56. Cammack KV, Taylor RM. Advanced neoplasms of head and neck. Treatment with combined radiation and chemotherapy. Rocky Mountain Med J. 1972;69(3):54-6. PMID 5057719.
57. Richards Jr GJ, Chambers RG. Hydroxyurea in the treatment of neoplasms of the head and neck: a resurvey. Am J Surg. 1973;126(4):513-8. doi: 10.1016/s0002-9610(73)80041-8, PMID 4743835.
58. Hussey DH, Abrams JP. Combined therapy in advanced head and neck cancer: hydroxyurea and radiotherapy. Prog Clin Cancer. 1975;6:79-86. PMID 1197760.
59. Lerner HJ. Concomitant hydroxyurea and irradiation: clinical experience with 100 patients with advanced head and neck cancer at Pennsylvania Hospital. Am J Surg. 1977;134(4):505-9. doi: 10.1016/0002-9610(77)90388-9, PMID 911036.
60. Saban N, Bujak M. Hydroxyurea and hydroxamic acid derivatives as antitumor drugs. Cancer Chemother Pharmacol. 2009;64(2):213-21. doi: 10.1007/s00280-009-0991-z, PMID 19350240.
61. Gwilt PR, Tracewell WG. Pharmacokinetics and pharmacodynamics of hydroxyurea. Clin Pharmacokinet. 1998;34(5):347-58. doi: 10.2165/00003088-199834050-00002, PMID 9592619.

62. Blasberg RG, Patlak C, Fenstermacher JD. Intrathecal chemotherapy: brain tissue profiles after ventriculocisternal perfusion. J Pharmacol Exp Ther. 1975;195(1):73-83. PMID 810575.
63. Engwa GA. Free radicals and the role of plant phytochemicals as antioxidants against oxidative stress-related diseases. Phytochemicals: source of antioxidants and role in Disease Prevention. BoD–books on. Demand. 2018;7:49-74.
64. Ghaisas MM, Navghare VV, Takawale AR, Zope VS, Deshpande AD. In vitro antioxidant activity of Tectona grandis Linn. Pharmacol Online. 2008;3:296-305.
65. Wilson DW, Nash P, Buttar HS, Griffiths K, Singh R, De Meester F et al. The role of food antioxidants, benefits of functional foods, and influence of feeding habits on the health of the older person: an overview. Antioxidants (Basel). 2017;6(4):81. doi: 10.3390/antiox6040081, PMID 29143759.
66. Smith MA, Perry G, Richey PL, Sayre LM, Anderson VE, Beal MF et al. Oxidative damage in Alzheimer's. Nature. 1996;382(6587):120-1. doi: 10.1038/382120b0, PMID 8700201.
67. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. Free Radic Biol Med. 1997;23(1):134-47. doi: 10.1016/s0891-5849(96)00629-6, PMID 9165306.
68. Weinbrenner T, Cladellas M, Isabel Covas MI, Fitó M, Tomás M, Sentí M et al. High oxidative stress in patients with stable coronary heart disease. Atherosclerosis. 2003;168(1):99-106. doi: 10.1016/s0021-9150(03)00053-4, PMID 12732392.
69. Prasad KN, Xie H, Hao J, Yang B, Qiu S, Wei X et al. Antioxidant and anticancer activities of 8-hydroxypsoralen isolated from wampeeClausenalansium (Lour.) Skeels peel. Food Chem. 2010;118(1):62-6. doi: 10.1016/j.foodchem.2009.04.073.
70. Fresco P, Borges FI, Diniz C, Marques MP. New insights on the anticancer properties of dietary polyphenols. Med Res Rev. 2006;26(6):747-66. doi: 10.1002/med.20060, PMID 16710860.
71. Rahman MM, Islam MB, Biswas M, Alam AK. In vitro antioxidant and free radical scavenging activity of different parts of Tabebuia pallida growing in Bangladesh. BMC Res Notes. 2015;8(1):1-9.
72. Al-Rimawi F, Rishmawi S, Ariqat SH, Khalid MF, Warad I, Salah Z. Anticancer activity, antioxidant activity, and phenolic and flavonoids content of wild Tragopogon porrifolius plant extracts. Evid Based Complement Alternat Med. 2016;2016:9612490. doi: 10.1155/2016/9612490, PMID 27999608.
73. Nagmoti DM, Khatri DK, Juvekar PR, Juvekar AR. Antioxidant activity free radical-scavenging potential of PithecellobiumdulceBenth seed extracts. Free Radic Antioxid. 2012;2(2):37-43. doi: 10.5530/ax.2012.2.2.7.
74. Bhaskar H, Balakrishnan N. In vitro antioxidant property of laticiferous plant species from Western Ghats Tamil Nadu, India. Int J Health Res. 2009;2(2):1-10.
75. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3, PMID 36810.
76. Lakhanpal P, DK R. Quercetin: a versatile flavonoid. Internet J Med Update. 2007;2(2):22-37.
77. Saeed N, Khan MR, Shabbir M. Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. BMC Complement Altern Med. 2012;12(1):221. doi: 10.1186/1472-6882-12-221, PMID 23153304.
78. ParamasivamRagavendran DS, Raj CA, Starlin T, Gopalakrishnan VK. Phytochemical screening, antioxidant activity of Aerva lanata (L.)-An Invitro study. Asian J Pharm Clin Res. 2012;5(2):77-81.
79. Duan X, Jiang Y, Su X, Zhang Z, Shi J. Antioxidant properties of anthocyanins extracted from litchi (Litchi chinenesisSonn.) fruit pericarp tissues in relation to their role in the pericarp browning. Food Chem. 2007;101(4):1365-71. doi: 10.1016/j.foodchem.2005.06.057.
80. Wang AN, Yi XW, Yu HF, Dong B, Qiao SY. Free radical scavenging activity of Lactobacillus fermentumin vitro and its antioxidative effect on growing–finishing pigs. J Appl Microbiol. 2009;107(4):1140-8. doi: 10.1111/j.1365-2672.2009.04294.x, PMID 19486423.
81. Usuki R, Engoh Y, Kaneda T. A simple and sensitive evaluation method of antioxidant activity by the measurement of ultraweakchemiluminescence. Nippon Shokuhin Kogyo Gakkaishi:198128(11):583-7.
82. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19. doi: 10.1097/WOX.0b013e3182439613, PMID 23268465.
83. Buettner GR. Superoxide dismutase in redox biology: the roles of superoxide and hydrogen peroxide. Anticancer Agents in Medicinal Chemistry (formerly current medicinal chemistry-anti-cancer agents). 2011;11(4):341-6.
84. Mahmoud SM, Paish EC, Powe DG, Macmillan RD, Grainge MJ, Lee AH et al. Tumor-infiltrating $CD8^+$ lymphocytes predict clinical outcome in breast cancer. J Clin Oncol. 2011;29(15):1949-55. doi: 10.1200/JCO.2010.30.5037, PMID 21483002.
85. Muniyandi K, George E, Mudili V, Kalagatur NK, Anthuvan AJ, Krishna K et al. Antioxidant and anticancer activities of Plectranthusstocksii Hook. Agric Nat Resour. 2017;51(2):63-73. doi: 10.1016/j.anres.2016.07.007.
86. Vijayakumar S, Bhuvaneshwari V, Sumathi A. Antioxidant and anticancer potential of methanolic leaf extract of MoringaconcanensisNimmo against human breast cancer cell line MCF-7. Int J Pharmacogn Phytochem Res. 2017;9(6):750-4.
87. Mardina V, Ilyas S, Halimatussakdiah H, Harmawan T, Tanjung M, Yusof F. Anticancer, antioxidant, and antibacterial activities of the methanolic extract from Sphagneticola trilobata (L.) JF pruski leaves. J Adv Pharm Technol Res:202112(3):222.
88. Mainasara MM, Abu Bakar MF, Md Akim A, Linatoc AC, Abu Bakar FI, Ranneh YKH. Secondary Metabolites, Antioxidant, and Antiproliferative Activities of Dioscorea bulbifera Leaf Collected from Endau Rompin, Johor, Malaysia. Evid Based Complement Alternat Med. 2021;2021:8826986. doi: 10.1155/2021/8826986, PMID 33505506.
89. Hazra B, Biswas S, Mandal N. Antioxidant and free radical scavenging activity of Spondias pinnata. BMC

Complement Altern Med. 2008;8(1):63. doi: 10.1186/1472-6882-8-63, PMID 19068130.
90. Arirudran B, Janani B, Rao UM. Evaluation of antioxidant and chemopreventive Potential of methanolic extracts of leaf of Aegle marmelosattributes towards Ductal Carcinoma studied in MCF-7 Cells. Int J Pharm Pharmacuetical Sci. 2019;11:21-5.
91. Gautam V, Sharma A, Arora S, Bhardwaj R, Ahmad A, Ahamad B et al. In-vitro antioxidant, antimutagenic and cancer cell growth inhibition activities of Rhododendron arboreum leaves and flowers. Saudi J Biol Sci. 2020;27(7):1788-96. doi: 10.1016/j.sjbs.2020.01.030, PMID 32565697.
92. Behera SK. Phytochemical screening and antioxidant properties of methanolic extract of root of Asparagus racemosus Linn. Int J Food Prop. 2018;21(1):2681-8. doi: 10.1080/10942912.2018.1560310.
93. Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem. 2007;102(3):771-6. doi: 10.1016/j.foodchem.2006.06.022.
94. Benzie IF, Strain JJ. [2]. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 1999;299:15-27. doi: 10.1016/s0076-6879(99)99005-5, PMID 9916193.
95. Halliwell B. The wanderings of a free radical. Free Radic Biol Med. 2009;46(5):531-42. doi: 10.1016/j.freeradbiomed.2008.11.008.
96. Oktay M, Gülçin İ, Küfrevioğlu Öİ. Determination of in vitro antioxidant activity of fennel Foeniculum vulgare seed extracts. LWT Food Sci Technol. 2003;36(2):263-71. doi: 10.1016/S0023-6438(02)00226-8.
97. Moein MR, Moein S, Ahmadizadeh S. Radical scavenging and reducing power of Salvia mirzayaniisubfractions. Molecules. 2008;13(11):2804-13. doi: 10.3390/molecules13112804, PMID 19015620.
98. Xiong Y, Wu X, Tetrastigmahemsleyanum RL. (Sanyeqing) root tuber extracts induces apoptosis in human cervical carcinoma HeLa cells. J Ethnopharmacol:2015165:46-53.
99. Paudel MR, Chand MB, Pant B, Pant B. Antioxidant and cytotoxic activities of Dendrobium moniliforme extracts and the detection of related compounds by GC-MS. BMC Complement Altern Med. 2018;18(1):134. doi: 10.1186/s12906-018-2197-6, PMID 29685150.
100. Silva MJD, Carvalho AJS, Rocha CQ, Vilegas W, Silva MA, Gouvêa CMCP. Ethanolic extract of Mimosa caesalpiniifolia leaves: chemical characterization and cytotoxic effect on human breast cancer MCF-7 cell line. S Afr J Bot. 2014;93:64-9. doi: 10.1016/j.sajb.2014.03.011.
101. Naz R, Roberts TH, Bano A, Nosheen A, Yasmin H, Hassan MN et al. GC-MS analysis, antimicrobial, antioxidant, antilipoxygenase and cytotoxic activities of Jacaranda mimosifolia methanol leaf extracts and fractions. PLOS ONE. 2020;15(7):e0236319. doi: 10.1371/journal.pone.0236319, PMID 32726328.
102. Khamsan S, Liawruangrath S, Teerawutkulrag A, Pyne SG, Garson MJ, Liawruangrath B. The isolation of bioactive flavonoids from Jacaranda obtusifolia HBK ssp. rhombifolia (GFW Meijer) Gentry. Acta Pharm. 2012;62(2):181-90. doi: 10.2478/v10007-012-0014-1, PMID 22750816.