



***In-Vitro* antioxidant activity of pharmacological effect and GC-MS analysis of bioactive compounds presents in *Catharanthus roseus* leaf extract**

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Abstract

The phytochemical analysis secondary metabolites *Catharanthus roseus* leaves methanolic and aqueous extract presences and absences. Leaves are utilized in the treatment of menorrhagia, stiffness, dyspepsia, heartburn, dysmenorrhea, diabetes, hypertension, feminine problems, antiallergic, calming, antimicrobial, antithrombotic, cardioprotective, antihelminthic, hypolipidemic, skin sicknesses, draining loose bowels, and antiviral properties. GCMS examination - Bioactive mixtures major Seven bioactive compounds parts are distinguished as Mono-inositol, Hexadecanoic corrosive, methyl ester, Hexadecanoic corrosive, 9-octadecanoic acid(Z)- methyl ester, Heptadecene-(8)- Carbonic corrosive (1), Octanoic corrosive, Eicosanoic corrosive, methyl ester, Icosanoic corrosive, 1,2-Benzenedicarboxylic corrosive, Squalene, Desmethoxy-vidoline individually. *In-vitro* anti-oxidant activity of (A) DPPH scavenging activity (B) H₂O₂ scavenging activity (C) Nitric oxide scavenging activity (D) FRAP assay. The FRAP assay also exhibit a constant increase in reducing ability with increase in the concentration which is indicative of the extract's antioxidant potential. In order for anticancer claims to be sufficient and yield the possibility of a future cancer treatment, *Catharanthus roseus* leaves extract must not harm non-cancerous cells.

Keywords: *Catharanthus roseus*, phytochemicals, GC-MS, *In-Vitro* antioxidant

Introduction

Catharanthus roseus is an important medicinal plant of apocynaceae family which contain variety of alkaloids and chemotherapeutic agents to treat various diseases like breast cancer, melanomas & lung cancer [1]. According to World Health Organization (WHO) approximately 80% of the World's population the herbal medicine. The herbal drugs are easy to intake, safe and reliable for human beings. Potential use of large plants as a source in estimated that 2,500–5,000 plant species medicinal property. Natural resources in important a new drugs. A sources or origins such as terrestrial plants, microorganisms, marine organisms, vertebrates, and invertebrates [2]. From time immemorial role of phytochemicals in treating and preventing a number of human diseases. Phytochemicals have been derived from the Greek word "phyto" meaning plant. Phytochemicals are biologically active, naturally occurring, non-nutritive chemical compounds found in plants having a protective and disease preventive activity. Plants produce such chemicals to safeguard themselves, but research reveals that they also have the ability to protect humans against diseases [3]. The subject of phytochemistry has been developed in recent years as a strict discipline, closely related to both natural product organic chemistry and plant biochemistry [4]. Plant metabolism is separate phytochemicals analysis of in two categories, namely, primary or secondary. Auxiliary constituents are the excess plant synthetics like alkaloids, terpenes, flavonoids, lignans, plant steroids, curcumines, saponins, phenolics, flavonoids,

and glucosides. Presently, natural examination has been for the most part zeroing in on segregation, portrayal, ID, and measurement of bioactive constituents and auxiliary metabolites [5]. Gas chromatography-mass spectrometry (GC-MS) is one such modern insightful method utilized in recognizable proof, discovery, and examination of the constituents. It includes GC-MS, by which complex combinations of plant-related mixtures might be isolated, distinguished, and measured [6]. In the current work, we have distinguished and affirmed the constructions of the dynamic constituents. *Catharanthus roseus* L. (G.) Don has a place with the Apocynaceae family. Nitty gritty phytochemical contemplates showed a few exceptionally glycosylated flavonoids, predominantly quercetin, kaempferol, and isorhamnetin subsidiaries. Different classes of phenolic compounds, to be specific benzoic acids, and phenylpropanoids have likewise been portrayed [7]. It is referred to for its uses as antileukemic and antimutagenic, cancer prevention agent, anti-diabetic, antimicrobial, for improving blood flow, as vasodilatory, transient depressor, to murder some intestinal parasites. It has gentle anti-toxin impacts and is antihypertensive [8]. Many exploration articles had been distributed revealing the natural exercises of these plants. The synthetic constituents of the plants are exceptionally affected by the variety in hereditary, topographical, and occasional factors just as the formative phases of the concerned plant and their parts/tissues. In contrast to engineered drugs, which normally contain a solitary dynamic compound focusing on a particular

medication target, plant concentrates may contain different dynamic fixings focusing on numerous medication targets. Therefore, taking account of these reports, the present study aims to quantify the polyphenols and flavonoids in the methanolic extract from leaves of *C. roseus*.

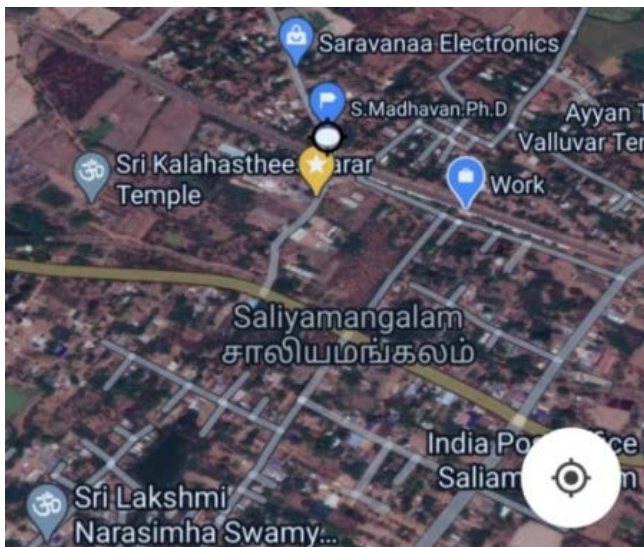
Materials and Methods

Plant Collection

The fresh leaves of *Catharanthus roseus* were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India.



Fig 1: *Catharanthus roseus* Leaves



Map 1: Study area

Phytochemical Studies

Secondary metabolites in the present studies were carried out on the plant sample revealed the presence of medicinally active constituents. Beneficial drugs and to improve the patient health.

Preparation of Extracts

The powdered plant tests of leaf (100 g) were utilized for progressive dissolvable extraction (500ml) with expanding request of polarities like methanol and Aqueous. By then it is kept in an orbital shaker at 190-220rpm for 48 hours. The

supernatant was gathered, sifted through Whatman No.1 filter paper and the concentrate were concentrated by a Rotary carafe evaporator at a particular temperature was utilized dependent on the dissolvable framework. Each time prior to extricating with the following dissolvable the buildup was dried completely to eliminate the dissolvable utilized. They got dried concentrate was then precisely gauged, put away in little vials at - 20°C and utilized for the accompanying investigations.

Phytochemical Screening

The preliminary phytochemical evaluation was carried out by using standard procedure [9-12].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110°C, and then maintained at the same temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C per minute and maintained for 9 min. The temperature of the injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected gradually in split mode as 10:1. The range of mass spectrum was set at 45-450 (mhz). The chemical constituents were identified by

GC-MS. The discontinuity examples of mass spectra were contrasted and those put away in the spectrometer information base utilizing National Institute of Standards and Technology Mass Spectral information base (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

Understanding of the mass spectrum of GC-MS was conducted using the mass spectral database of the National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The range of the obscure segment was contrasted and the range of the realized segments put away in the NIST library. The name, sub-atomic weight, and structure of the parts of the test materials was determined.

In-vitro Anti-Oxidant Assays

DPPH Free Radical Scavenging Assay

The DPPH radical-scavenging activity of the test extracts was examined using the modified method by Brand-Williams *et al.* [13]. Leaf extracts of different concentrations (50e200 mg/mL) were mixed with an equal volume of methanolic solution of DPPH (Sigma Aldrich). The mixture was allowed to react at room temperature in dark for 30 min. Ascorbic acid (1 mg/mL (50e200 mg/mL)) was used as positive control. After 30 min the absorbance was measured at 517 nm and converted into percentage of antioxidant activity using the following equation.

$$\% \text{ of inhibition} = [A_0 - A_1 / A_0] * 100$$

Where A₀ = Absorbance of control.
A₁ = Absorbance of test.

Hydrogen peroxide scavenging assay

The H₂O₂ scavenging activities for both the leaf extracts were assayed by the modified method [14]. Different concentrations of plant leaf extracts (50e200 mg/mL) and ascorbic acid at different concentrations (50e200 mg/mL) of (1 mg/mL) were added to 40 mM H₂O₂ solution prepared in phosphate buffer. The absorbance of H₂O₂ at 230 nm was determined after 10 min. The percentage of H₂O₂ scavenging by the extracts and standard (H₂O₂) was calculated as follows.

$$\% \text{ of scavenged } [H_2O_2] = [A_0 - A_1 / A_0] * 100$$

Where A₀ = Absorbance of control.
A₁ = Absorbance of test

Nitric Oxide Radical Scavenging Assay

The nitric oxide (NO) scavenging activity was determined using the method described by Parul *et al.* [15]. 10 mM sodium nitroprusside was incubated with 100 mL leaf extract for 60 min at 30 °C. After incubation, 100 mL of griess reagent was added. The absorbance of the chromatophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylendiamine was measured at 562 nm. Ascorbic acid (1 mg/mL) was at the same concentration was taken as standard.

$$\% \text{ NO scavenged} = [A_0 - A_1 / A_0] * 100$$

Where A₀ = Absorbance of control.

A₁ = Absorbance of test.

Ferric Reducing Power (FRAP) Assay

The reducing power was determined by Benzie and Strain [16] with slight modifications. Various concentrations of plant leaf extracts (50e200 mg/mL) were mixed with phosphate buffer and 2 mM potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. TCA was added to the mixture which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution was mixed with distilled water and freshly prepared FeCl₃ solution (0.5 mL) and the absorbance was recorded at 700 nm using UV-Visible spectrophotometer (Thermo scientific evolution -201 series). Ascorbic acid (50e200 mg/mL) was used as positive control. Reducing capacity was calculated as follows:

$$\% \text{ increase in reducing power} = [A_{\text{test}} / A_{\text{blank}} - 1] * 100$$

Where A_{test} = Absorbance of test solution.

A_{blank} = Absorbance of blank.

Statistical Analysis

All assays were performed in triplicate. Mean and standard deviation (SD) was examined for all assays. The results were expressed as mean ± SEM of three experiments. One way ANOVA with Dunnett's test was followed to compare each concentration with positive control to analyze level of statistical significance. P < 0.05 were considered statistically significant using Graph pad PRISM v.8.0.

Results and Discussion

Phytochemical Analysis

The plant extracts of *C. roseus* prepared in five different solvents were evaluated for the presence of different phytochemicals and the result obtained are presented in perusal of the results reveal that all the five extracts, showed the presence of alkaloids, phenols and flavonoids [17]. Methanolic extract showed the maximum number of phytoconstituents, followed by the ethanol and aqueous extract. Glycosides were detected in all the extracts, except petroleum ether extract. Terpenoids were detected in both methanolic and chloroform extracts. Saponins were detected in the aqueous extract only.

Table 1: Qualitative Phytochemicals analysis of *Catharanthus roseus* extract leaves

S. No	Analysed Phytochemicals Factor	Aqueous	Methanolic
1.	Tannin	+	+
2.	Saponin	-	+
3.	Flavonoids	+	+
4.	Steroids	+	-
5.	Alkaloids	+	-
6.	Polyphenol	+	+

Indications: "+" means positive activity, "-" means negative activity

The characteristic phytochemical constituents are Flavonoids, Steroids, Phenols, Terpenoids, proteins and Glycoside are establish in the concentrates of *Catharanthus roseus* flower [18]. Demonstrated gentle to direct movement and better anthelmintic action when contrasted with methanol separate.

GC-MS Analysis *Catharanthus roseus* Methanolic Extract Leaves

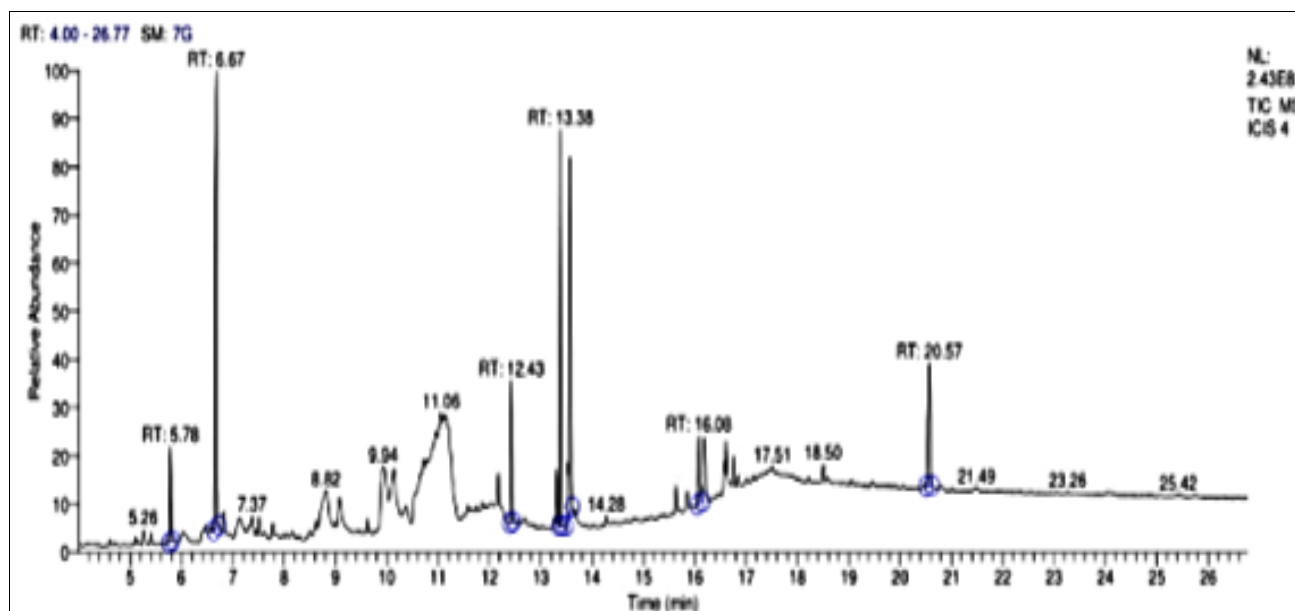


Fig 2: GC-MS CHROMATOGRAM OF *Catharanthus roseus* METHANOLIC EXTRACT LEAVES

Table 2: GCMS analysis- Bioactive compounds

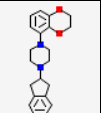
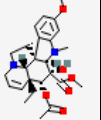
S. No.	Name of the Compounds	Molecular weight g/mol	Molecular Formula	Retention time	Peak Area %
1.	4H-Pyran-4-one	144	C ₆ H ₈ O ₄	5.78	4.73
2.	5Hydroxymethylfurfural	126	C ₃ H ₆ O ₃	6.67	30.86
3.	n-Hexadecanoic acid	256	C ₁₆ H ₃₂ O ₂	12.43	6.58
4.	Phytol	296	C ₂₀ H ₄₀ O	13.38	17.17
5.	9,12,15Octadecatrienoic acid	278	C ₁₈ H ₃₀ O ₂	13.57	23.64
6.	2,20-Cycloaspido-spermidine-3-carboxylic acid Vindoline	336	C ₂₁ H ₂₄ N ₂ O ₂	16.08	4.95
7.	Aspidospermidine-3-carboxylic-acid vindoline	400	C ₂₂ H ₂₈ N ₂ O ₅	20.57	12.09

The common mixtures in 1-1-Diethoxy-Ethane, 3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl), Octadecanoic corrosive, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, cis-(CAS), Benzene, 1-ethyl-2,3-dimethyl-(CAS), α-L-Galactopyranose, 6-deoxy-, cyclic 1,2:3,4-bis(methylboronate), 1-Hexadecanol (CAS), 11-Octadecenal (range dissents) ^[19]. (CAS), 2-Hexadecanol (CAS), 7-Methoxy-2, 2-dimethyl-2H-1-benzothiopyran, 1-Octadecene [20]. (CAS), Lucenin 2, 1,3-diformyl-2-chloro-5-isopropylbenzene, 6-Octadecenoic corrosive, 1-Octadecene (CAS), Pentadecanoic corrosive, 14-methyl-, methyl ester

(CAS), Hexadecanoic corrosive, ethyl ester ^[21]. (CAS), 6-Octadecenoic corrosive, 9-Octadecenoic Acid (Z)-, Ethyl Ester, Bacteriochlorophyll-c-stearyl, Heptaethylene glycol monododecyl ether, 1HCyclopropano [3, 4] benz [1, 2-e]azulene-4a,5,7b,9,9a(1aH)- pentol,3-[(acetyloxy)methyl]-1b,4,5,7a,8,9-hexahydro-1,1,6,8-tetramethyl-9,9a-diacetate, [1aR 1aa,1ba,4aa,5a,7aa,7ba,8a,9a,9aa], Stigmasterol, Methyl 7-ethyl-10-hydroxy-11-hydroxy(18o)-3,11-dimethyl-2,6-tridecadienoate, Stigmast-5-en-3-ol, (3a)-(CAS), ^[22].

Table 3: GC-MS Analysis of Activities/Uses of bioactive compounds of *Catharanthus roseus*

S. No.	Name of the Compounds	Structure	Biological Activity / Uses
1	4H-Pyran-4-one		Antioxidant, Antimicrobial, Anti-inflammatory and Antioxidant ^[23]
2	5Hydroxymethylfurfural		Antioxidant, Antiproliferative activity
3	n-Hexadecanoic acid		Antioxidant, Hypocholester-olemic, Nematicide, Pesticide, Lubricant, Anliandrogenic, Flavor ^[24]
4	Phytol		Hypocholesterolemic, Antimicrobial, Anticancer, Diuretic, Anti-inflammatory
5	9,12,15Octadecatrienoic acid		Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge ^[25]

6	2,20-Cycloaspido-spermidine-3-carboxylic acid Vindoline		Anticancer, Anti-inflammatory
7	Aspidospermidine-3-carboxylic acid vindoline		Anticancer, Antimicrobial

Retention Time (20.57). Aspidospermidine-3-carboxylic acid vindoline. RT (13.57). 9, 12, 15 Octadecatrienoic acid. RT (6.67) 5Hydroxymethylfurfural. Molecular bioactive compounds. GC-MS assessment of cement showed presence of glucose, fructose, sucrose, maltose and xylose and exhibited its degree to be of consistent importance particularly as plant polymer based excipient and covering material in drug things.

In-vitro Cancer Prevention Agent Concentrate of *Catharanthus roseus*

Cell reinforcements are a substance that cooperate with and kills free extremists, hence keeping harm of cells from revolutionaries. Cancer prevention agents are otherwise called "free extreme scroungers" and body makes a portion of the cell reinforcements to kill free revolutionaries [26-30]. These cell reinforcements are called endogenous cancer prevention agents. Notwithstanding, the body depends on

outer (exogenous) sources, basically the eating routine, to get the remainder of the cell reinforcements it needs [31]. Capacity of methanol concentrate of *C. roseus* to rummage free revolutionaries was surveyed utilizing 1, 1-diphenyl-2-picrylhydrazyl revolutionary (DPPH) [32]. Methanol concentrate of *C. roseus* has ability to search free extremists as displayed by the information by lessening the stable DPPH (1, 1-diphenyl-2-picrylhydrazyl) revolutionary to the yellow shaded 1, 1-diphenyl-2-picrylhydrazine [33, 34, 35]. Limit of DPPH decrease increments with expanding centralization of the concentrate as announced Superoxide anion revolutionary is probably the most grounded ro among the free revolutionaries and get changed over to other destructive receptive oxygen species, for example, hydrogen peroxide and hydroxyl extremist which are harming proteins in the cells which brings about ongoing illnesses.

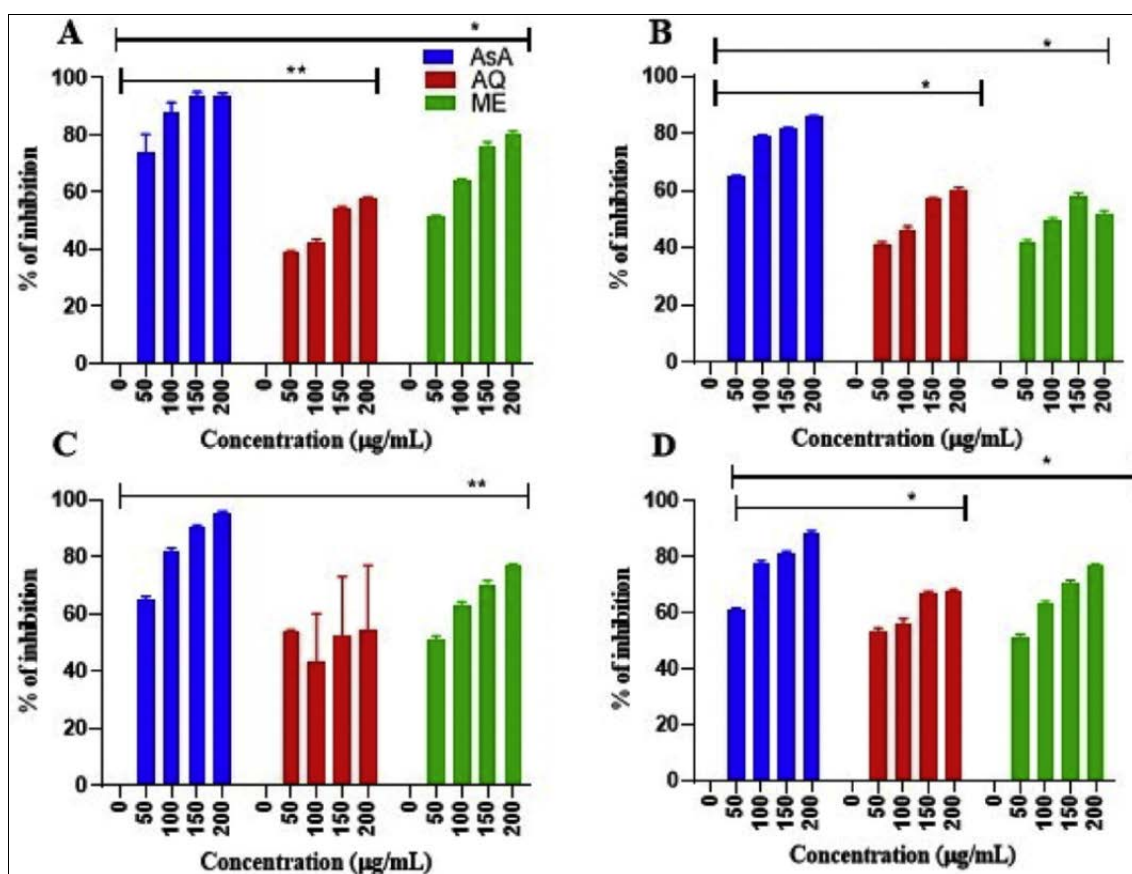


Fig 3: *In-vitro* antioxidant activity of aqueous and methanolic leaf extracts of *Catharanthus roseus*. (A) DPPH scavenging activity (B) H₂O₂ scavenging activity (C) Nitric oxide scavenging activity (D) FRAP assay. Values are expressed as Mean \pm SEM (n = 3). One-way ANOVA followed by Dunnett's test was employed to compare each concentration with positive control. *Statistical significance at p < 0.05; ** statistical significance at p < 0.01. AsA-Ascorbic acid (Positive control); AQ-Aqueous; ME-MeOH.

The maximum inhibition of superoxide radical. In the all-out cancer prevention agent action, ABTS^{•+} is a blue chromophore delivered by the response among ABTS and

potassium persulfate and within the sight of the plant separate or ascorbic acid, ABTS[•] Action radical gets reduced and the remaining radical cation concentration after

reaction with antioxidant compound was then quantified same kind of result in ABTS+• radical cation scavenging activity, which was concentration dependent [36, 37]. Rummaging of hydroxyl revolutionary is a significant cancer prevention agent movement as a result of high reactivity of the OH extremist which empowers it to respond with a wide scope of particles found in living cells, like sugars, amino acids, lipids and nucleotides. Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells.

Summary and Conclusion

Phytochemicals secondary metabolites. The presence of phytoconstituents make the plant useful for treating different diseases and have a possible of providing useful drugs to cure human diseases [38]. The strength of *Catharanthus roseus* was contrasted and glibenclamide, a standard antidiabetic drug. It shows that *Catharanthus roseus* exhibited little less hypoglycemic activity than glibenclamide [39]. An enzymic activity of glycogen synthase, glucose-6-phosphate-dehydrogenase, succinate dehydrogenase and malate dehydrogenase was improved in diabetic rats after treatment with extract of *Catharanthus roseus* due to increased metabolism of Glucose [40, 41]. Investigation of these bioactive mixtures may yield Anti-provocative, Anticancer, against ligament, antiulcer and antibacterial medications. This investigation will improve fundamental comprehension of the unrefined medication recognizable proof plan valuable in customary consideration rehearses and could prompt opposite pharmacology by which society would get home grown medications at a moderate expense. GC-MS analysis major bioactive compounds 9, 12, 15 Octadecatrienoic acid biological activity of Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge. 20-Cycloaspido-spermidine-3-carboxylic acid Vindoline anticancer activity. These exogenous antioxidants are commonly called dietary antioxidants. Cell reinforcements advantage the body by killing and eliminating the free revolutionaries from the circulatory system, Fruits, vegetables and grains are rich wellsprings of dietary antioxidants. Oxidative pressure the result of an irregularity of supportive of oxidants and cancer prevention agents in the organic entity and is a critical wonder in ongoing illnesses. Oxidative pressure is currently recognized to be related with in excess of 100 sicknesses, just as with the ordinary maturing measure, particularly individuals determined to have Alzheimer's illness. Cell reinforcements are personally engaged with the avoidance of cell harm the normal pathway for malignancy, maturing, and an assortment of infections. A very good *in vitro* membrane stabilization effect and protein denaturation effect showed that the plant extract possessed anti-inflammatory and anti-arthritis properties which has been seen with other species of plants. *In-vitro* anti-oxidant activity of maximum and minimum value. Hence, it might help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of ant diabetic drugs.

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Conflict of Interest

The authors stated that no conflicts of interest.

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