Determination of Bioactive Compounds Using GC-MS Analysis And Evaluation of Antioxidant Activity of *Catharanthus Roseus* Leaves Extract

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ABSTRACT

The aim of the study was to assess the different phytochemical profiles and the GC-MS analysis of the extracts of *Catharanthus roseus* leaves. The whole extract of *Catharanthus roseus* leaves showed the presence of tannin, saponin, steroids, terpenoids, alkaloids, flavonoids, triterpenoids, polyphenol, glycoside and coumarins, while anthocyanins were missing. Anthroquinone was contained in the ethanol and hydroethanol extract. The robe *Catharanthus* leaf extract had a significant concentration of polyphenol, flavonoid, tannin and terpenoids. GC-MS analysis of the leaves of *Catharanthus roseus* showed the presence of 20 compounds. The compounds identified have many biological properties. Antioxidant activity sieved by DPPH, total antioxidant activity, hydrogen peroxide, ABTS (2.2'-azino-bis- 3 -ethylbenzthiazolin- 6 -sulfonic acid) and nitrogen oxide scavenging activity at various concentrations and ascorbic acid as standard antioxidant.

Keywords: *Catharanthus roseus,* Phytochemicals, antioxidant, Qualitative, Quantitative and GCMS.

DOI Number: 10.14704/NQ.2022.20.15.NQ88378 **NeuroQuantology2022;20(15): 3760-3771**

INTRODUCTION

Medicinal plants have been used as a remedy for human diseases for centuries (Nostro et al., 2008; Arokiyaraj et al., 2008). Many plants in India are used as medicines because of their medicinal properties. The plant kingdom still contains many plant species which contain substances with medical values which have yet to be discovered. In-depth studies on the undesirable properties of these medicinal plants to establish a good correlation between biomarkers and stores are essential to frost the efficacy and quality of medicinal plants. Interest has increased recently. in the use of natural packaging of leaves and fauna due to their natural, inexpensive and lower by-products (Ahmad et al., 2008; Chellaram and Edward, 2009). Factory-based natural constitutions can be derived from any part of the factory, such as canoe, leaves, flowers, roots, fruits, seeds, etc. (Gordon, 2001).

The medicinal activities of plants characterized by certain species or groups of plants are reliable with the model, that the mixture of secondary products in a particular plant is fiscally different (Wink et al., 1999). Spectrometric and chromatographic analysis could provide the necessary preliminary information on the selection of extracts from raw plants with properties potentially useful for other chemical and pharmacological studies. In recent years, the SMGC has established itself as a central technological metabolic profile for plant and uncultivated species (Kell et al., 2005; Janakiraman et al., 2012; Sahaya Sathish et al., 2012). GC-MS is one of the best useful methods for the quantitative and qualitative detection of sample components. The technology allows the separation of volatile components with good sensitivity and selectivity and provides structural information on the compounds (Van Bramer and Goodrichs, 2015; Keller and Fabbri, 2012). Therefore, this study aimed to find the bioactive compounds of the phytochemical compounds present in the *Catharanthus roseus.* The qualitative and

eISSN 1303-5150

quantitative phytochemical screening, gas chromatography and mass spectrometry analysis and evaluation of antioxidant activity by various in vitro models were done.

MATERIALS AND METHOD.

Collection of plant materials

The Whole plant of *Catharanthus roseus* were collected from Chengalpet District, Tamil Nadu, India.

Preparation of extract

The powder was used for extraction with, aqueous, ethanol and hydroalcoholic extract of *Catharanthus roseus* for 24 hours and obtained semi solid mass after complete elimination of solvent under reduced pressure. The extracts were stored in refrigerator and used for further experiments.

Preliminary phytochemical analysis

Preliminary phytochemical screening was carried out by using standard procedure Sofowara (1993), Trease and Evans (1989) and Harborne (1973). The total phenol contents were estimated by the method of Kim *et al*., (2003). The total flavonoid was determined by the method of Katasani (2011). The total tannins estimated by Bajaj and Devsharma (1977) method. Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

GC MS Analysis

GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013). The mass spectrum was interpreted with the aid of the database and the unknown component was compared with the spectrum of the known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes, 2013).

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992). The antioxidant activity of hydro-alcoholic extract of *Catharanthus roseus* was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.* (1999). Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964). Hydrogen peroxide scavenging activity of the extract was estimated by method of Zhang (2000).

The antioxidant effect of the hydroalcholic extract of *Catharanthus roseus* was studied using ABTS (2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolourisation assay according to the method of Re *et al*. (1999).

Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically/ mean ± standard deviation.

RESULTS AND DISCUSSION

Results of the present study to examine the phytochemical analysis of aqueous, ethanol and hydro-ethanolic extract of *Catharanthus roseus* leaves powder. All extracts of *Catharanthus roseus* leaves showed the presence of tannin, saponin, steroids, terpenoids, alkaloids, flavonoids, triterpenoids, polyphenol, glycoside and coumarins while anthocyanins was absent. Anthroquinone was present in ethanol and hydroethanolic extract. Emodins was present in aqueous and hydroethanolic extract**.** A significant concentration of polyphenol, flavonoid, tannin and terpenoids were present in *Catharanthus roseus* leaves extract (Table 1-2).

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Table 1: Phytochemicals analysis of different extracts of *Catharanthus roseus*

S. No	Phytochemicals	Extract		
		Aqueous	Ethanol	Hydro-ethanolic
1	Tannin	+	++	++
2	Saponin	++	+	++
3	Flavonoids	++	++	++
4	Steroids	+	+	++
5	Terpenoids	+	++	++
6	Triterpenoids	+	+	++
7	Alkaloids	+	+	+
8	Anthroquinone	-	+	++
9	Polyphenol	++	++	++
10	Glycoside	+	+	++
11	Coumarins	++	+	++
12	Emodins	+	-	+
13	Anthocyanins	-	-	-

(-) Absent, (+) Present and (++) high concentration

Table 2: Quantitative phytochemical analysis of *Catharanthus roseus*

S. No	Phytochemicals	Result (mg/gm)
1	Total phenol	213.21±7.75
2	Flavonoids	156.30±3.48
3	Tannin	72.16±2.04
4	Terpenoids	58.48±1.67

Values expressed as Mean ± SD for triplicates

Thenmozhi and Rajan, (2015) have been assessed the bioactive components of *Psidium guajava* leaves by qualitative and quantitatively. The saponin, tannin, phlobatannins, steroids, flavonoids, triterpenoids, terpenoids, carbohydrate, glycoside and polyphenol present in ethanolic and aqueous extract of *Psidium guajava* leaves. The significant amount of Phenol, Flavonoids, Tannin, and Saponin present in *Psidium guajava* leaves. The outcomes of this work offer a platform of using extract of *Psidium guajava* leaves an herbal alternative for numerous diseases. The restorative effects of medicinal plants due to the presence of various phytochemicals (Stray, 1998).

Identification of bioactive compounds in *Catharanthus roseus* by GC MS analysis

Twenty compounds were identified in hydroalcholic extract of *Catharanthus roseus* using GC-MS technique. The identified compounds are presented in Table 3. The prevailing compounds are Undecane, 2-Pentadecanone, 6,10,14-trimethyl, Octadecanoic acid, Methyl este, 9-Octadecenoic acid, 1,2-Benzenedicarboxylic acid, 9,12-Octadecadienoyl chloride, 2-Hexadecen-1-ol, 3,7,11,15-Tetram and Hexadecanoic acid, methyl ester were found in this *Catharanthus roseus* The presence of various bioactive compounds justifies the use of the plant for various ailments by traditional practitioners. The biological activities of the selected compounds are represent in Table 4.

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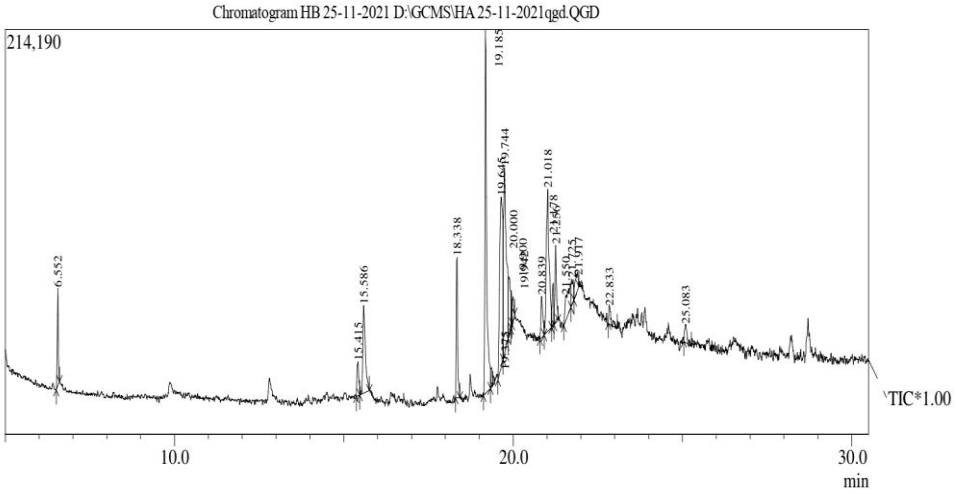
Fig. 1:



Chromatogram of *Catharanthus roseus*

Table 1: Identification of active compounds in *Catharanthus roseus* using GCMS

Peak	R. Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	6.552	2.86	6.08	$C_{11}H_{24}$	156	Undecane
2	15.415	1.77	2.12	$C_{16}H_{33}Cl$	260	Hexadecane, 1-Chloro
3	15.586	6.37	5.43	$C_{20}H_{26}O_4$	330	Phthalic acid, di-(1-hexen-5-yl) ester
4	18.338	5.29	8.72	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-trimethyl
5	19.185	18.48	22.62	$C_{19}H_{38}O_2$	298	Octadecanoic acid, Methyl este
6	19.375	0.86	0.96	$C_{15}H_{23}NO_3Si$	293	Pentanoic acid, 2-[(phenylmethoxy)imino]
7	19.645	15.09	10.59	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid
8	19.744	15.67	11.58	$C_{16}H_{22}O_4$	278	1,2-Benzenedicarboxylic acid
9	19.900	3.19	3.38	$C_8H_{18}O$	130	2,4,4-Trimethyl-1-pentanol
10	19.942	0.83	2.29	$C_4H_4O_2$	84	2-Propynoic acid, methyl ester
11	20.000	0.90	1.30	$C_5H_{10}ClNO_4$	183	L(+)-Glutaminic acid hydrochlo
12	20.839	2.27	2.57	$C_{11}H_{20}Cl_2O_2$	254	Dichloroacetic acid, nonyl ester
13	21.018	11.68	8.84	$C_{18}H_{31}ClO$	298	9,12-Octadecadienoyl chloride,
14	21.178	1.86	2.64	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol, 3,7,11,15-Tetram
15	21.256	3.44	4.83	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester
16	21.550	3.75	1.55	$C_{22}H_{42}O_2$	338	2-Heptadec-5"-EN-1"-yloxy tetrah
17	21.725	1.46	1.65	$C_{16}H_{12}D_2$	208	Anthracene, 9-(ethyl-2,2-D2)
18	21.917	1.72	0.75	$C_9H_{16}O_2$	156	6-Heptenyl acetate

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19	22.833	1.22	1.05	$C_{14}H_{42}O_5Si_6$	458	Silicone polymer
20	25.083	1.31	1.06	$C_{24}H_{72}O_{12}Si1_2$	888	Silicate anion tetramer
		100.00	100.00			

Table 2: Biological activity of compounds identified in *Catharanthus roseus* using GCMS

Peak	R. Time	Name of the compounds	Biological activity**
1	6.552	Undecane	Antimicrobial, carcinogenic activity
2	18.338	2-Pentadecanone, 6,10,14-trimethyl	Hypocholesterolemic, antioxidant, antimicrobial and lubrication activity
3	19.185	Octadecanoic acid, Methyl este	Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic activity
4	19.645	9-Octadecenoic acid	Anticancer, antimicrobial Anemiagenic, Insectifuge, Antiandrogenic, Dermatitigenic activity
5	19.744	1,2-Benzenedicarboxylic acid	Antimicrobial, Antifouling activity
6	21.018	9,12-Octadecadienoyl chloride	Anti-inflammatory, Insectifuge, Hypocholesterolemic, Cancer preventive, Nematicide, Hepatoprotective, Antihistaminic, Antieczemic, Antiacne, 5-Alpha reductase inhibitor
7	21.178	2-Hexadecen-1-ol, 3,7,11,15-Tetram	Antimicrobial, Cancer-preventive, Anti-inflammatory, Analgesic, Fungicide activity
8	21.256	Hexadecanoic acid, methyl ester	Antibacterial and antifungal activity

**Source: Dr. Duke's phytochemical and ethnobotanical databases [Online database].

The spectrum profile of GC-MS confirmed the presence of main components with their retention time. The heights of the peak show the relative concentrations of the components present in the extracts. In comparison of the mass spectra of the constituent with the NIST library, the phytoconstituents were characterized and identified. Kumari Ranjana *et al*. (2018) explored the phytochemicals present in the methanol extract of bark, flower, leaf and seed of *Madhuca indica* by GC-MS analysis. The important phytochemicals present in Leaf, Seed and Bark were Octylcyclohexane, E-14- Hexadecenal, Hexaylcyclohexane, Dibutyl Phthalate and Pentadecan-8-one, 8-Octadecanone. GC-MS is a key technological tool for secondary metabolites profiling in plant species (Merlin *et al.,* 2009; Janakiraman *et al*., 2012).

***IN VITRO* ANTIOXIDANT ACTIVITY**

DPPH radical scavenging activity

In the DPPH test, the antioxidant could reduce the stable radical DPPH to the yellow 1, 1 diphenyl 1, 2 picrylhydrazine .The 2, 2 -Diphenyl 1, 1 - Picrylhydrazine molecule is characterized as a stable free radical due to the relocation of the replacement electron over the entire molecule. The proton transfer reaction of the DPPH-Free-Radical results in a decrease in absorption at 517 nm, followed by a common spectrophotometer in the visible range. The effects of antioxidants on DPPH are thought to be due to their hydrogen donation capacity (Sindhu and Abhram, 2006) Table 1 and Figure 1 show the radical DPPH

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acid activity of the *Catharanthus roseus* extract and are compared with ascorbic acid. The radical rinsing activity of the DPPH was directly proportional to the concentration of the extract of *Catharanthus roseus*. The semi-maximum inhibitory concentration (IC_{50}) of ascorbic acid (47.19 µg / ml) and of *Catharanthus roseus* extract (52.21 µg / ml) were each. The entire present study depended on the concentration of the extract as a% of the inhibition.

Table 3: DPPH radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Catharanthus roseus	**Std. (Ascorbic acid)**
20	20.94±0.86	24.04±0.81
40	36.42±0.95	42.62±0.97
60	63.02±1.54	65.21±1.36
80	77.78±1.23	81.23±1.43
100	84.69±1.68	94.17±1.35
IC_{50} (µg/ml)	52.21	47.19

Values expressed as Mean ± SD for triplicate

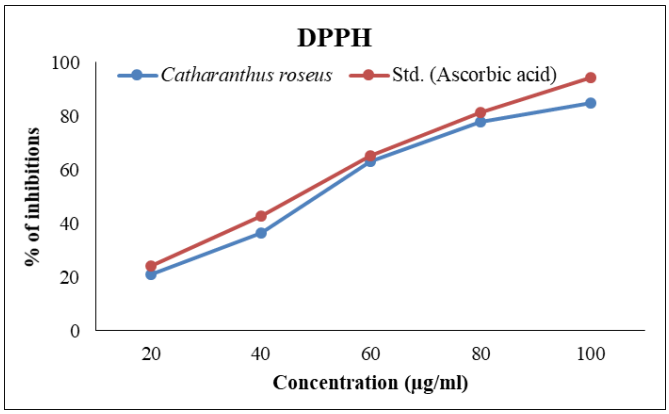

Figure 1: DPPH radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Total antioxidant capacity

The antioxidant activity of the extracts was based on the phosphomolybdenum method according to the Prieto et al procedure. (1999) evaluated. The test is based on the reduction of Mo (VI) - Mo (V) by the extract and the subsequent formation of a green phosphate complex Mo (v) at 695 nm, where the antioxidant activity is expressed as the number of equivalents of ascorbic acid. Table 2 and Figure 2 showed the total antioxidant activity of *Catharanthus roseus* extract compared to ascorbic acid. Total antioxidant activity was increased with the increase in the concentration of C*atharanthus rose* extract. The semi-maximum inhibitory concentration (IC_{50}) of ascorbic acid (49.33 µg / ml) and of *Catharanthus roseus* extract (53.74 µg / ml) was or. The entire present study depended on the concentration of the extract as a% of the inhibition.

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Table 2: Total antioxidant activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Catharanthus roseus	**Std. (Ascorbic acid)**
20	18.14±1.23	25.31±1.17
40	38.61±1.07	42.82±1.25
60	57.17±1.35	59.07±1.19
80	70.88±1.28	75.31±1.38
100	82.27±1.54	91.35±2.04
IC_{50} (µg/ml)	55.74	49.33

Values expressed as Mean ± SD for triplicate

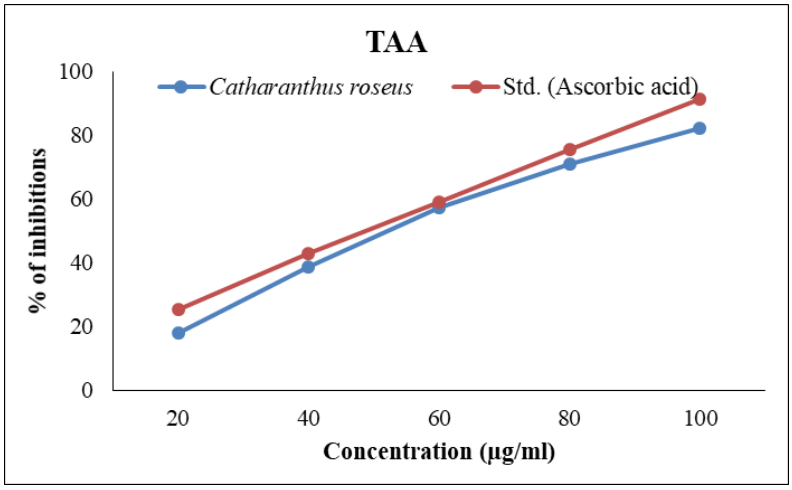

Figure 2: Total antioxidant activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

ABTS radical scavenging activity

Table 2 and Figure 2 noted the activity of radical recovery of the extract of Catharanthus roseus in ABTS and compared to ascorbic acid. ABTS's radical scavenger activity increased with the increase in the concentration of *Catharanthus roseus* extract. The semi-maximum inhibitory concentration (IC_{50}) of ascorbic acid (46.57 µg / ml) and of *Catharanthus roseus* extract (59.64 µg / ml) was or. It reflects the ability of hydrogen phonic antioxidants in the extract to eat the radical ABTS cation. The entire present study depended on the concentration of the extract as % of the inhibition. These results suggest that samples dictated by invasive can prevent or delay the *in vitro* formation of radical species associated with oxidative stress, and can play an important role in protecting against damage to the functions of the membrane. Similar observations between the phenolic components and the $ABTS^{\bullet+}$ recovery activity have been reported for several plant extracts, including broccoli (Puertas *et al*. 2005).

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Table 5: ABTS radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Catharanthus roseus	Std. (Ascorbic acid)
20	18.21±0.78	24.48±1.13
40	31.39±1.13	45.83±1.29
60	50.71±1.08	64.36±1.31
80	67.34±1.23	79.27±1.28
100	83.83±1.87	93.87±1.61
IC_{50} (µg/ml)	59.64	46.57

Values expressed as Mean ± SD for triplicate

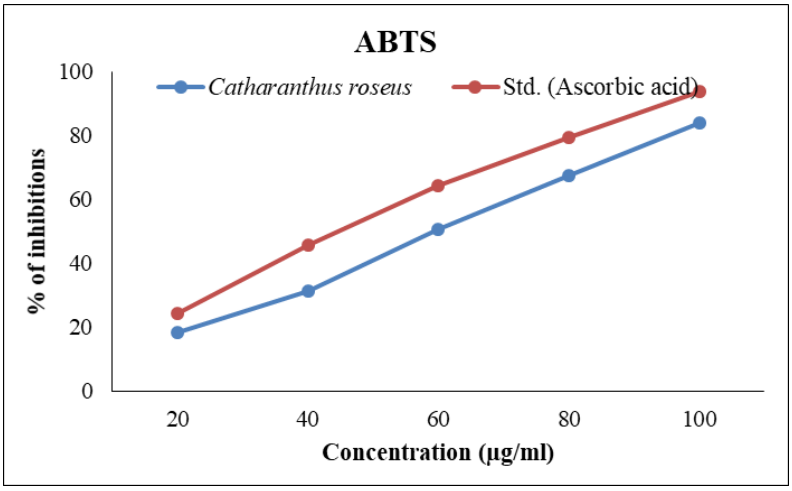

Figure 3: ABTS radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Nitric oxide radical scavenging activity

Nitrogen oxide (NO0) released by sodium nitroprusside (SNP) has a strong NO^+ symbol which can change the structure and function of many cellular components. *Catharanthus* leaf extract showed good NO acid activity, which resulted in a reduction in the nitrite concentration in Assay Medium. NO cleaning capacity depended on concentration. *Catharanthus roseus* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO, which was released into the reaction medium compared to standard SNP ascorbic acid $NO^·$ toxicity increases when it reacts with superoxide to form peroxynitritanion (ONOO-), which is a potentially potent oxidizing agent, which can decompose to produce OH and NO_2 (Proteggente et al., 2002). Table 2 and Figure 2 noted the activity of the radical nitrogen oxide exchange of the extract of *Catharanthus roseus* and compared to ascorbic acid. The radical rinsing activity with nitrogen oxide was directly proportional to the concentration of the extract of *Catharanthus roseus*. The semi-maximum inhibitory concentration (IC_{50}) of ascorbic acid (46.70 µg / ml) and of *Catharanthus roseus* extract (53.98 µg / ml) were each. The entire present study depended on the concentration of the extract as a% of the inhibition.

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Table 6: Nitric oxide radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Catharanthus roseus	Std. (Ascorbic acid)
20	19.43±1.28	24.45±1.17
40	39.49±1.15	47.33±1.04
60	57.99±1.69	63.32±1.51
80	70.53±2.01	77.74±1.88
100	87.77±2.16	92.47±2.05
IC_{50} (µg/ml)	53.98	46.70

Values expressed as Mean ± SD for triplicate

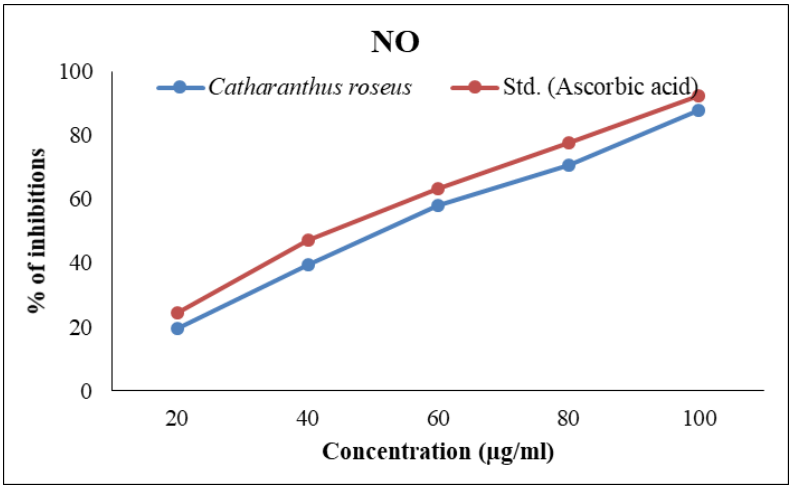

Figure 4: Nitric oxide radical scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Hydrogen peroxide scavenging activity

The natural origin of H_2O_2 in air, water, human bodies, plants, microorganisms and food is in low concentration. It is quickly broken down into oxygen (O_2) and water (H2O) and can produce hydroxyl radicals (OH), which can trigger lipid peroxidation and damage DNA. Table 2 and Figure 2 have determined the activity of recovering hydrogen peroxide from the extract of *Catharanthus roseus* and compared to ascorbic acid. The activity to recover hydrogen peroxide was directly proportional to the extract concentration of *Catharanthus roseus*. The extract from *Catharanthus roseus* has recovered the hydrogen peroxide, which is due to the presence of phenolic groups that give electrons to the hydrogen peroxide and could thus neutralize it in H_2O. The maximum inhibitory concentration (IC_{50}) of ascorbic acid was 50.58 µg / ml and the extract of *Catharanthus roseus* was 58.17 µg / ml. Overall, this study was the percentage inhibition based on the concentration of the dependent extract.

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Table 7: Hydrogen peroxide scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

Concentrations (µg/ml)	% of inhibitions	
	Catharanthus roseus	Std. (Ascorbic acid)
20	17.23±1.24	23.02±1.48
40	35.18±1.13	40.85±1.22
60	52.89±1.45	59.03±1.31
80	70.36±1.37	74.61±1.53
100	81.82±1.84	93.74±2.35
IC_{50} (µg/ml)	58.17	50.58

Values expressed as Mean ± SD for triplicate

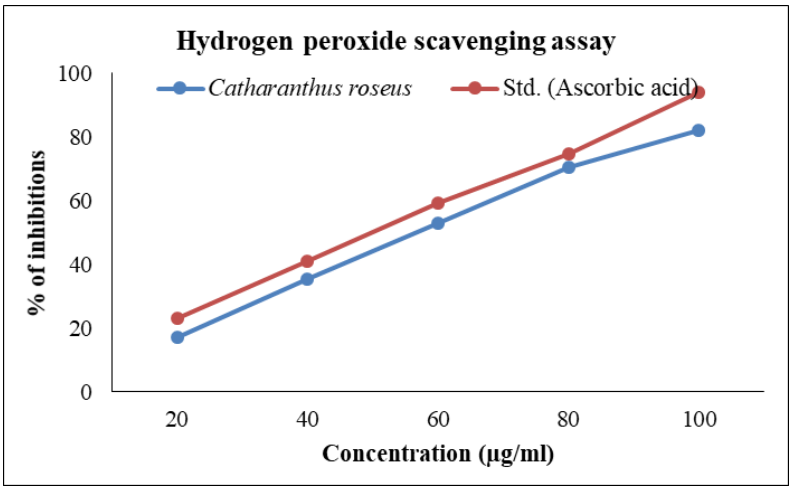

Figure 5: Hydrogen peroxide scavenging activity of *Catharanthus roseus* extract and compared with standard Ascorbic acid

CONCLUSION

The result of this study shows that extract from *Catharanthus rose* leaves have a rich source of plant substances and good antioxidant activities. Experimental evidence of the extract as a natural antioxidant for its ability to recover reactive types of oxygen and nitrogen and to protect organisms from oxidative damage, and could therefore be effective against oxidative stress, including cardiovascular diseases, cancer, diabetes, etc.,

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