

# In vitro antioxidant assay of selected aqueous plant extracts and their polyherbal formulation

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## Abstract

The consumption of selected plant extracts in Ayurveda, naturopathy, the antioxidant potential of the aqueous extract of *Vinca rosea* (VR), *Gymnema sylvestre* (GS), *Tinospora cordifolia* (TC) and *Embllica officinalis* (EO) and their mixture (PHF) of Indian origin was investigated by using *in vitro* models like superoxide, hydroxyl radical scavenging activity and lipid peroxide inhibition assay. The effects were related with standard (ascorbic acid), a known antioxidant. Various phytoconstituents identified in the above selected plants extracts were poly phenols, flavonoids, terpenoids, tannins, alkaloids. The terpenoids were stated to possess the scavenging properties against the free radicals. The presence of these phytoconstituents in selected plants might be responsible for antioxidant activity; ascorbic acid is used as a standard.

**Keywords:** Polyherbal formulation (PHF), Anti-oxidant activity, Ascorbic acid (A.A)

## 1. Introduction

In the last few years, there has been a significant growth in the arena of herbal treatment. It is getting propagated in emergent countries due to its natural origin and lesser side effects[1][2]. It is categorized in the olden Indian system of medicine (Ayurveda) as Rasayana, a group of plant derivative remedies that develop overall physical and mental health and put off diseases by rejuvenating the body in incapacitated conditions.

Free radicals has been involved in the connection of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes etc[3] and the compounds that can scavenge free radicals have great prospective in improving these disease processes[4]. Our body has in-built mechanism to decrease the free radical induced damage by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase and others such as vitamin E, exogenously administered ascorbic acid etc. At times these protective mechanisms were found to be not adequate when related to the insult produced to the body. Therefore the quest for exogenous antioxidants is persistent.

To support the usage of selected plant extracts in Ayurveda, naturopathy, the antioxidant potential of the aqueous extract of *Vincarosea* (VR), *Gymnema sylvestre* (GS), *Tinospora cordifolia* (TC) and *Embllica officinalis* (EO) of Indian origin was examined. The objective of this work was to assess the antioxidant activity of aqueous extracts of above listed herbs (VR, GS, TC and EO) and their combination (PHF) by *in vitro* studies and relate them with ascorbic acid, a known antioxidant.

## 2. Materials and Methods

### 2.1 Plant extracts

The polyherbal formulation under research was obtained as extracts from Laila Impex, Vijayawada, Andhra Pradesh, India.

### 2.2 Determination of superoxide radical scavenging activity:

#### 2.2.1 Riboflavin photoreduction method

Superoxide scavenging activity of aqueous extract of *Vincarosea* (VR), *Gymnema sylvestre* (GS), *Tinospora cordifolia* (TC) and *Embllica officinalis* (EO) and their combination (PHF) were determined by Mccord[5] which depends on light induced superoxide production by riboflavin and the equivalent decline of nitrobluetetrazolium. 0.1 ml of different

concentrations of plant extracts, and 0.1 ml of 6 $\mu$  Methylenediaminetetra acetic acid containing NACN, 0.1 ml of 50  $\mu$ M nitrobluetetrazolium, 0.05 ml of 2  $\mu$ M of riboflavin were added to a test tube, and final volume was made upto 3ml using phosphate buffer.

Then the assay tubes were evenly irradiated with an incandescent light (40 Watts) for 15 minutes and thereafter the optical densities were measured at 560 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of superoxide produced was assessed by comparing the absorbance values of control and experimental tubes.

### 2.2.2 Calculation of percentage inhibition

The percentage inhibition of superoxide generated by the extract was calculated using the formula:

$$\text{Inhibitory ratio} = \frac{(A_0 - A_1) \times 100}{A_n}$$

Where,  $A_0$  is the absorbance of control;

$A_1$  is the absorbance with addition of plant extract/ascorbic acid.

### 2.3 Determination of hydroxyl radical scavenging activity:

#### 2.3.1 Deoxyribose degradation method

Hydroxyl radical scavenging activity of aqueous extract of *Vinca rosea* (VR), *Gymnema sylvestre* (GS), *Tinospora cordifolia* (TC) and *Embllica officinalis* (EO) and their mixture (PHF) was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe<sup>2+</sup>/EDTA/H<sub>2</sub>O<sub>2</sub> system (Fenton reaction). The hydroxyl radical assaults deoxyribose, which finally results in the development of thiobarbituric acid reacting substances (TBARS)[6]. Fenton reaction mixture consisting of 200  $\mu$ l of 10 mM of Ferrous sulphate (FeSO<sub>4</sub>.7 H<sub>2</sub>O), 200 $\mu$ l of 10mM EDTA and 200  $\mu$ l of 10 mM 2-deoxyribose and was mixed with 1.2 ml of 0.1 M phosphate buffer (pH 7.4) and 200  $\mu$ l of plant extracts. Thereafter, 200  $\mu$ l of 10 mM H<sub>2</sub>O<sub>2</sub> was added before the incubation at 37°C for 4 hrs. Then, 1ml of this fenton reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 0.8% thiobarbituric acid and 1.5 ml of 20% acetic acid. The entire volume was then made upto 5 ml by adding distilled water and kept in an oil bath at 100°C for 1 hour. The mixture is allowed to cool, then added 5 ml of 15:1 v/v butanol-pyridine mixture. Subsequent vigorous shaking, the tubes were centrifuged at 4000 rpm for 10 min and the absorbance of the organic layer comprising the thiobarbituric acid reactive substances was measured at 532 nm. A control was prepared using 0.1 ml of vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of hydroxyl radicals by the extracts/compound was determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay.

### 2.4 Determination of lipid peroxidation inhibition activity

#### 2.4.1 Induction of lipid peroxidation by Fe<sup>2+</sup>/ascorbic acid

Inhibition of lipid peroxidation of aqueous extract of *Vinca rosea* (VR), *Gymnema sylvestre* (GS), *Tinospora cordifolia* (TC) and *Embllica officinalis* (EO) and their combination (PHF) was evaluated[7]. Rat liver tissue weighing 10 gm was homogenized with a polytron homogenizer in ice-cold tris-HCl buffer to produce a 25% w/v homogenate. The homogenate was centrifuged at 4000rpm for 10 min. An aliquot of supernatant 0.1 ml was mixed with 0.1 ml of plant extract of different concentrations, followed by addition of 0.1 ml of potassium chloride (30mM), 0.1 ml ascorbic acid (0.06mM ) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) and were incubated for 1 hour at 37°C. The reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20% acetic acid (PH 3.5). The final volume was then made upto 4 ml by adding distilled water and kept in an oil bath at 100°C for 1 hour. The mixture is cooled, add 1 ml of distilled water and 5 ml of 15:1 v/v butanol-pyridine mixture. Following vigorous shaking, the tubes were centrifused at 4000 rpm for 10 min and the absorbance of the organic layer having the thiobarbituric acid reactive substance (TBARS) was measured at 532 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract/ascorbic acid. The percentage inhibition of lipid peroxidation by the extract was determined.

## 3. Results

The aqueous extracts of *Vinca rosea*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Embllica officinalis* and PHF scavenged the superoxide produced by photo reduction of riboflavin. The selected aqueous extracts and their polyherbal formulation (30, 60, 90, and 120  $\mu$ g of concentrations) produced dose dependant inhibition of superoxide radical activity. The ascorbic acid at 30, 60, 90 and 120  $\mu$ g was also found to prevent superoxide radicals in a subsequent manner and the mean values of five samples for each concentration were represented in table: 3.1, 3.2, and 3.3 The quantity of the extracts and their PHF needed for 50% scavenging of superoxides (table: 3.1) was found to be 214.09  $\mu$ g (VR), 176.02  $\mu$ g

(GS), 206.64 µg (TC), 228.78 µg (EO) and 188.10 µg (PHF)

The quantity needed for the same effect by the known antioxidant, ascorbic acid was 110.71 µg. This indicated that the aqueous extracts of the VR, GS, TC, EO and PHF possess comparable antioxidant activity (fig: 1).

Deprivation of deoxyribose intervened by hydroxyl radicals produced by Fe<sup>3+</sup>/Ascorbate/EDTA/H<sub>2</sub>O<sub>2</sub> system was found to be inhibited by these extracts. All the extracts and their PHF at 10, 25, 50, 75,100, 125 and 150 µg levels scavenged the hydroxyl radicals in a dose dependant manner. Ascorbic acid at 10, 25, 50, 75, 100, and 125 µg was also found to produce dose dependant inhibition of hydroxyl radicals and the mean value of five samples for each concentration were shown in (table: 2). The amount of these extracts needed for 50% inhibition were found to be 123.23 µg (VR), 110.41 µg (GS), 117.96 µg (TC), 143.07 µg (EO) and 107.02 µg (PHF)

The amount of the ascorbic acid desired for 50% inhibition of hydroxyl radical was found to be 87.45 µg. This indicated that aqueous extracts of selected herbs and their PHF possess comparable antioxidant activity(fig: 2).

Lipid peroxides caused by the induction of Fe<sup>2+</sup>/Ascorbate on rat liver homogenate was found to be inhibited by the selected herbs and their polyherbal formulation. All the above extracts and their PHF at concentrations of 20, 50, 100, 150, 200 and 300 µg produced inhibition of lipid peroxide in a subsequent manner and the mean values of five samples for each concentration were represented in (table: 3). The IC<sub>50</sub> of the extracts and their PHF was found to be 252.41µg (VR), 231.13 µg (GS), 188.97 µg (TC), 307.34 µg (EO) and 201.70 µg (PHF). The IC<sub>50</sub> of the ascorbic acid was found to be 165.88 µg. This showed that the aqueous extracts VR, GS, TC, EO and their PHF possess equivalent antioxidant activity (fig: 3).

**Table 1: Percentage Inhibition of Superoxide Radicals Using Photo Reduction Method**

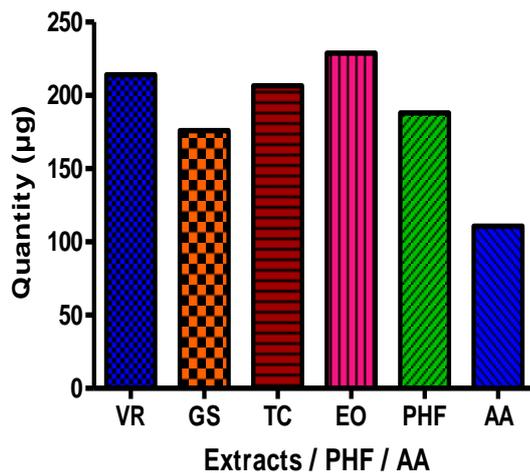
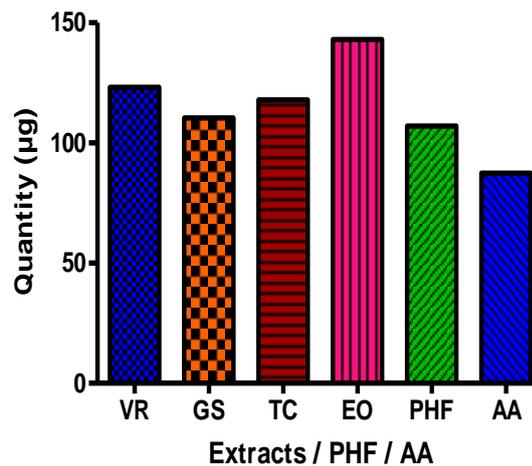
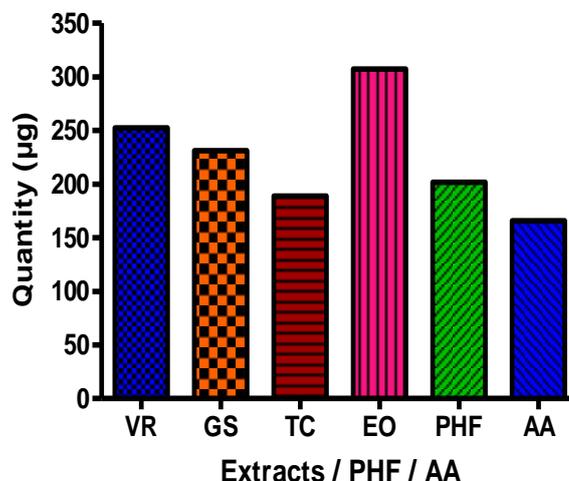
Extracts	Quantity (µg)								
	15	30	60	90	120	180	240	300	360
<i>Vinca rosea</i>	3.11±0.81	8.58±2.24	17.39±3.55	28.47±6.21	35.61±6.81	45.54±6.30	56.49±6.82	62.64±7.68	-
<i>Gymnema sylvestre</i>	5.72±1.22	13.38±2.68	21.77±5.96	34.35±6.54	51.26±8.13	58.92±8.27	63.49±8.42	68.54±7.89	-
<i>Tinospora cordifolia</i>	0.45±0.08	0.65±0.16	15.77±2.96	20.65±4.54	37.26±6.13	45.92±6.27	59.49±6.42	67.54±8.69	-
<i>Emblica officinalis</i>	7.45±0.08	9.47±1.16	28.78±4.96	29.65±4.54	34.86±5.63	46.47±5.27	50.49±6.15	57.54±8.14	-
Polyherbal formulation	4.35±1.02	10.49±2.75	19.58±4.12	32.84±5.87	46.25±7.63	53.47±7.42	60.43±6.97	67.84±7.61	-
Ascorbic acid	3.39±0.39	16.86±1.54	27.67±2.64	40.07±5.81	53.81±6.46	-	-	-	-

**Table 2: Percentage Inhibition of Hydroxyl Radical Using Deoxyribose Method**

Extracts	Quantity (µg)								
	10	25	50	75	100	125	150	175	200
<i>Vinca rosea</i>	7.88±1.21	16.89±1.91	28.28±3.12	38.31±3.89	43.49±4.10	49.32±4.52	56.38±5.15	-	-
<i>Gymnema sylvestre</i>	4.89±0.67	14.54±1.89	23.98±2.50	37.88±3.48	53.39±4.12	57.89±5.10	67.50±5.36	69.20±6.89	-
<i>Tinospora cordifolia</i>	12.72±1.91	17.89±2.10	25.8±3.50	35.93±4.89	43.15±5.75	54.30±5.78	63.17±6.23	66.78±6.38	-
<i>Emblica officinalis</i>	4.48±1.21	9.40±1.09	16.28±1.13	24.39±2.28	33.49±3.15	46.79±3.45	55.31±5.71	58.38±5.23	-
Polyherbal formulation	6.34±1.13	15.18±1.78	26.78±2.69	39.85±3.78	49.54±3.93	55.86±4.74	65.13±5.24	-	-
Ascorbic acid	7.75±2.26	27.10±2.50	32.64±5.01	47.78±2.15	52.78±4.28	67.00±6.75	-	-	-

**Table 3: Percentage Inhibition of Lipid Peroxidation Using Thiobarbituric Acid Method**

Extracts	Quantity (µg)									
	20	50	100	150	200	250	300	350	400	450
<i>Vinca rosea</i>	5.32±0.98	17.81±2.22	26.84±2.75	34.58±3.58	39.19±4.95	47.98±4.48	58.52±5.91	-	-	-
<i>Gymnema sylvestre</i>	2.53±0.49	3.41±0.56	12.48±1.78	25.79±2.41	45.32±4.28	56.89±7.91	66.76±7.19	-	-	-
<i>Tinospora cordifolia</i>	15.58±1.30	25.79±2.56	31.89±3.21	43.40±5.91	54.78±7.50	64.91±8.10	66.16±6.98	-	-	-
<i>Emblica officinalis</i>	1.28±0.25	2.97±0.38	14.68±2.21	26.35±5.61	38.41±6.24	45.60±4.60	47.79±5.41	52.50±6.18	63.78±7.21	-
Polyherbal formulation	8.79±0.87	17.54±1.59	28.71±2.40	40.84±4.59	52.85±5.83	60.41±5.86	67.84±6.74	-	-	-
Ascorbic acid	5.79±1.01	23.41±1.56	37.44±2.98	45.37±4.46	56.54±4.98	-	-	-	-	-

Fig.1: IC<sub>50</sub> Values of Extracts / PHF/ AA in Inhibition of Superoxide radicalFig.2: IC<sub>50</sub> Values of Extracts / PHF/AA in Inhibition of Hydroxyl radicalFig.3: IC<sub>50</sub> Values of Extracts / PHF/AA in Inhibition of Lipid peroxidation

#### 4. Discussion

Several phytochemicals possessing polyphenolic structures are advocated as nutraceuticals to supplement food for better health care during recent years. Most of them are claimed to possess antioxidant activity. Ayurveda and naturopathy the medical systems indigenous to India advocates the use of plants extracts/ mixtures of extracts for treating various disorders apart from others from times immemorial in humans without preclinical evidence which is required to make the systems popular and scientific. The claimed usefulness of herbs in several disorders might be due to their antioxidant activity. To support the use of the selected plant extracts in herbal mixture and in Ayurveda and naturopathy, the antioxidant potential of the aqueous extract of *Vinca rosea*(VR), *Gymnema sylvestre*(GS), *Tinosporacordifolia*(TC) and *Emblica officinalis*(EO) of Indian origin was investigated in comparison with the known antioxidant ascorbic acid(AA) following *In vitro* studies. The antioxidant activity of ascorbic acid was well established[8]. The order of potency of the selected herbal extracts, PHF and AA in 50% inhibition of superoxide radical, hydroxyl radical and lipid peroxidation were found to be AA>GS>PHF>TC>VR>EO, AA>PHF>GS>TC>VR>EO and AA> TC>PHF>GS> VR>EO respectively.

Herbal drugs comprising antiradical constituents are acquiring importance in inhibition and treatment of stress related disorders. The free radical scavengers like polyphenolics are well identified for their therapeutic activity in disorders such as cancer, diabetes and skin[9].

Earlier reports on the above plants indicated the presence of compounds representing polyphenols, flavanoids, terpenoids, tannins, alkaloids etc[10]-[14]. The terpenoids and flavonoids having glucosidic linkage were likely to be extracted into aqueous extracts. The terpenoids were reported to scavenge the free radicals, some types of reactive oxygen,

hydroxylic groups, peroxides and superoxide radicals[15]. In experimental studies, terpenoids have stopped the occurrence of cancer in many tissues including lung, breast, colon, stomach, prostate, pancreas, liver and skin[16]. The occurrence of these structures in our selected herbs might be responsible for the comparable antioxidant activity with that of known antioxidant ascorbic acid.

## 5. Conclusion

The synthetic compounds are replaced with natural antioxidants(because of implications for human health) may be beneficial. In the current study analysis of free radical scavenging activity showed that aqueous extracts of *Vinca rosea*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Embllica officinalis* and PHF can be the potent source of natural antioxidants. Antioxidant activity of *Vinca rosea*, *Gymnema sylvestre*, *Tinospora cordifolia*, *Embllica officinalis* and PHF may be due to existence of various phytoconstituents as polyphenols, flavanoids, terpenoids, tannins, alkaloids; so selected plants can be used as potent antioxidants.

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