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Free radical scavenging activity of *Soymida febrifuga* leaves by DPPH, nitric oxide and reducing power methods

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Abstract
Rohan, *Soymida febrifuga* plant belonging to family meliaceae. The bark of this plant has been proven to have many pharmacological activities. In this experiment the antioxidant activity of the plant leaves were studied using different techniques like DPPH radical scavenging, Nitric oxide scavenging and reducing power. The methanol, water and total aqueous extracts were used for the study. In DPPH method, Methanolic extract shows highest antioxidant activity. The IC_{50} Value of methanol extract is found 20.56 as compared with standard ascorbic acid IC_{50} Value 16.74. Lower IC_{50} Value indicates greater antioxidant activity. In nitric oxide scavenging method, Methanolic extract shows highest antioxidant activity. % inhibition on NO is found 78.98 for methanol extract at conc. 100 ug/ml as compared with standard ascorbic acid % inhibition on NO is found 90.52 for methanol extract at conc. 100 ug/ml. Higher % inhibition Value indicates greater antioxidant activity. In case of reducing power method, Methanolic extract shows highest antioxidant activity. Maximum absorbance 0.911 is found for methanol extract at conc. 1000 ug/ml as compared with standard ascorbic acid maximum absorbance is found 0.973 for methanol extract at conc. 1000 ug/ml. Higher absorbance value indicates greater antioxidant activity.

Keywords: *Soymida febrifuga*, antioxidant, DPPH, ascorbic acid

Introduction
Oxidative stress can cause a wide range of diseases including Alzheimer's and Parkinson's disease, diabetes, cardiovascular disease, rheumatoid arthritis, cancer and other diseases). Antioxidants are compounds that are capable of slowing or preventing the oxidation of other molecules. Antioxidants can terminate chain reactions by removing free radicals and oxidation reaction blocking others by being oxidized themselves. Due to this, antioxidants such as polyphenols and flavones that are often reducing factors. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are very effective in neutralizing free radicals and therefore they are used as inhibitors of lipid peroxidation to stabilize food containing fat. Carcinogenicity and toxicity of these synthetic antioxidants have been reported. Consequently, the use of synthetic antioxidants is limited. In recent years, researchers are trying to replace them with natural antioxidants such tocopherols, flavonoids and rosemary for food preservation (Frutos and Hernandez-Herrero, 2005) [15]. Fruits and vegetables are reported to contain large amount of antioxidants, so people who consume these products have a lower risk of the relevance of free radical diseases such as heart disease and neurological disorders. Moreover, medicinal plants have been shown to have antioxidant activity by virtue of the presence of phenolic diterpenes, flavonoids, tannins and phenolic acids. Medicinal plants have been regarded as a source of bioactive natural compounds. Antioxidants have the ability to protect the body against damages caused by oxidative stress.
Soymida febrifuga is a tall tree belonging to family meliaceae; commonly known as Indian redwood, bastrol cedar. Pharmacologically the plant is of great importance in the ethano-medicinal use. It contains some important constituents like in bark lupeol, sitosterol, methyl angolensate, leaves contains Quercetin, rutin and fruits abundantly contains tetraterpenoids. The ethno botanical use in treatment of diarrhea, dysentery and fever, as a bitter tonic in general debility, treatment of rheumatic swelling, in gargles, vaginal infection etc.

Material and Method
Collection of plant material and extraction: The leaves of the plants were collected from forest department office at Amravati. The plant was authenticated at botany department of VMV. The leaves were dried in shade and were powdered, in the soxhlet apparatus it was kept for successive extraction with Pet.

Ether, chloroform, methanol and water. A part of powdered leaves were directly extracted with water to get total aqueous extract.

Estimation of Free Radical Scavenging Activity

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of cotelle *et al* 19 with some modification. In brief, 3ml reaction mixture containing 200 µl of DPPH (100µm in methanol) and 2.8ml of different extract of *Soymida febrifuga* (at various concentration 10-100 µg/ml) in methanol was incubated at 37 for 30min and absorbance of the mixture was read at 517nm using (Shimatzdu) spectrophotometer.

The percentage inhibition of DPPH radical was calculated by comparing the results of the test with those of the control (not treated with extract) using the formula-

$$\text{Percentage Inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \times 100$$

Nitric Oxide Scavenging Method

Principle

Measurement of nitric oxide generated by sodium nitroprusside with the help of Griess reagent is as described previously. At physiological pH in aqueous solution sodium nitroprusside impulsively generates nitric oxide. Griess reagent plays important role in the measurement of nitric ions formed by the coupling of nitric oxide and oxygen. Nitric oxide scavenger's comets with oxygen radical and results in production of nitric oxide

Preparation of Sodium Nitroprusside Solution (5mM)

An accurately weighed 0.148 gm of sodium nitroprusside as transferred in 100 ml volumetric flask, it was dissolved in standard phosphate buffer, and volume was up to 100 ml with phosphate buffer.

Preparation of Griess Reagent

Accurately weighed sulphanilamide (0.5 gm) and N-(1-Napthyl) ethylene dihydrochloride were added to it. This was then dissolved in standard phosphate buffer; finally volume was adjusted with standard phosphate buffer.

Preparation of Standard Phosphate Buffer_(0.025 M, 7.4pH)

An accurately weighed 3.40 gm of potassium dihydrogen phosphate and 3.55 gm of anhydrous disodium hydrogen phosphate both previously dried at 110 to 130 ^{0}C for 2 hours, dissolved in sufficient water to produce 1000 ml.

Preparation of Stock Test Extracts Solutions

An accurately weighed quantity of extracts (10 mg) was dissolved in respective solvents (100 ug/ml).

Preparation of working test extract solutions

The aliquot portions of stock solution of test extract solutions were diluted appropriately with representative solvents to obtain a concentration range of 10-100 ug/ml.

Procedure

Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentration of alcoholic and aqueous extracts of dried leaves of *Soymida febrifuga* A. Juss dissolved in methanol and incubated at room temperature for 180 min. The same reaction mixture without the extract of the sample but with equivalent amount of phosphate buffer served as the control. After incubation period 0.5ml Griess reagent {1% sulphanilamide, 2% H_3PO_4 and 0.1% N-(naphthyl) ethylenediamine hydrochloride NEDA} was added to equivalent amount of sample. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with NEDA was measured at 546 nm for the determination.

Determination of Reducing Power

Extracts (100-1000ug) in 1ml of distilled water mixed with 2.5ml of phosphate buffer (0.2m, pH6.6) and 2.5ml potassium ferricyanide(1%) and then the mixture was incubated at 50 C for 30 min.Afterwards,2.5ml of trichloroacetic acid (10%) was then centrifuged at 3000rpm for 10min. Finally, 2.5ml of upper layer solution was mixed with 2.5ml distilled water and 0.5ml ferric chloride (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated reducing power.

Result and Discussion

Free Radical Scavenging Activity (Inhibition of DPPH radical)

Table 1: DPPH radical scavenging activity of each extract

Sr. No	Extr-acts	Concentration (µg/ ml) and % inhibition						IC 50 (µg/ml)
		10	20	40	60	80	100	
(1)	ME	28.34 ±0.28	48.64 ±0.94	68.87 ±0.93	87.81 ±0.54	90.98 ±0.64	90.67 ±0.42	20.559
(2)	WA	20.21 ±0.79	26.29 ±0.21	30.72 ±0.25	42.72 ±0.36	60.14 ±0.75	72.56 ±0.97	70.224
(3)	TA	25.85 ±0.96	45.25 ±0.85	55.42 ±0.79	71.81 ±0.76	83.98 ±0.58	84.36 ±0.56	36.088
(4)	Vit. C	30.95 ±0.56	59.73 ±0.64	91.66 ±0.93	93.43±0.88	93.84 ±0.25	95.18 ±0.49	16.742

ME-methanol, WA-water, TA-total aqueous, Vit. C-vitamin C

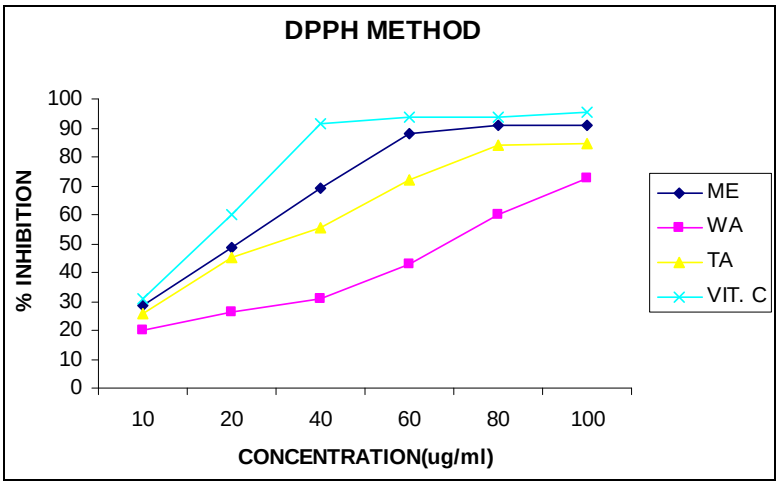

Fig 1: The effect of each extract on the accumulation of DPPH radical

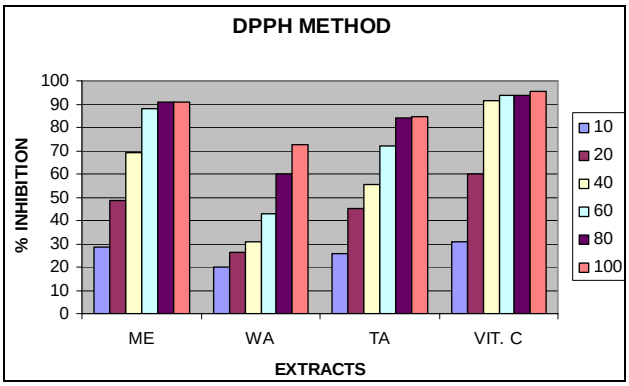

Fig 2: The effect of each extract on the accumulation of DPPH radical

DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in its absorbance at 517 nm. This is induced by antioxidants.

Table no. 1 illustrates a significant decrease in the conc. of DPPH radical due to the scavenging ability of soluble solids in the various extracts and the standard ascorbic acid as a reference compound presented the highest activity at all concentrations.

Inhibition of Nitric Oxide radical

Table 2: Nitric oxide scavenging activity of each extract

Sr. No.	Extracts	CONCENTRATION (ug/ml)					
		10	20	40	60	80	100
1	ME	14.79±0.56	29.64±0.12	33.75±0.29	42.01±0.33	49.79±0.15	78.97±0.10
2	WA	21.65±0.65	30.54±0.73	34.11±1.02	50.52±0.76	58.45±0.60	71.44±0.75
3	TA	19.61±.052	25.78±0.25	31.59±0.98	45.45±0.77	56.88±0.23	70.31±0.47
4	Vit. C	23.82±0.25	40.64±0.78	51.94±0.69	62.78±0.52	75.12±0.75	90.52±0.92

ME-methanol, WA-water, TA-total aqueous, Vit. C-vitamin C

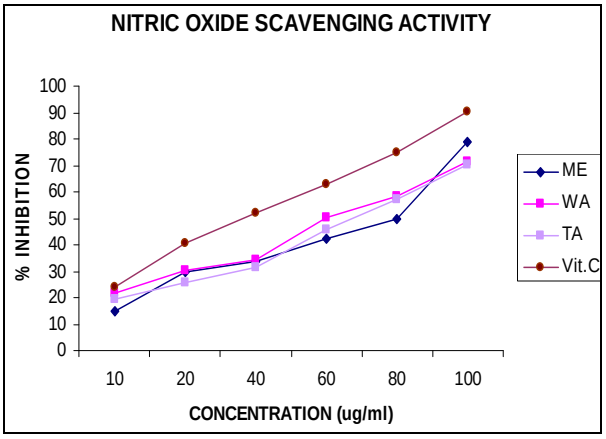

Fig 3: Production of nitrite from solution of 10 mM Sod. nitroprusside in the presence of extracts

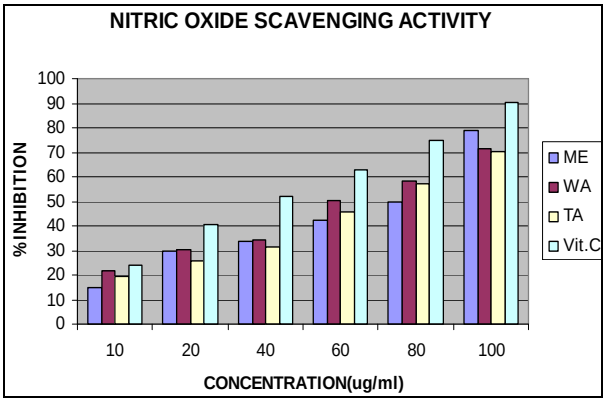

Fig.4: Production of nitrite from solution of 10 mM Sod. Nitroprusside in the presence of extracts

The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylenediamine was read at 546 nm

and referred to the absorbance of standard solution of ascorbic acid treated in the same way with Griess reagent.
Table no.2 illustrates a significant decrease in the conc. of nitric oxide radical with an increase in the concentration of extracts.

Reducing power method

Table 3: Absorbance of each extract by RP method

Sr. No.	Extracts	Conc. ug/ml	Absorbance at 700 nm
1.	Methanol	100	0.232
		200	0.381
		400	0.591
		600	0.783
		800	0.885
		1000	0.911
2.	water	100	0.212
		200	0.257
		400	0.345
		600	0.421
		800	0.485
		1000	0.573
3.	Total Aqueous	100	0.035
		200	0.136
		400	0.266
		600	0.392
		800	0.558
		1000	0.668
4.	Vit. C	100	0.361
		200	0.477
		400	0.625
		600	0.793
		800	0.884
		1000	0.973

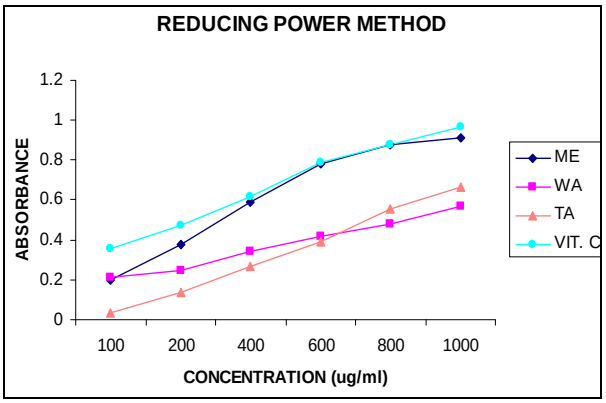

Fig 5: Inhibition by each extract by RP method

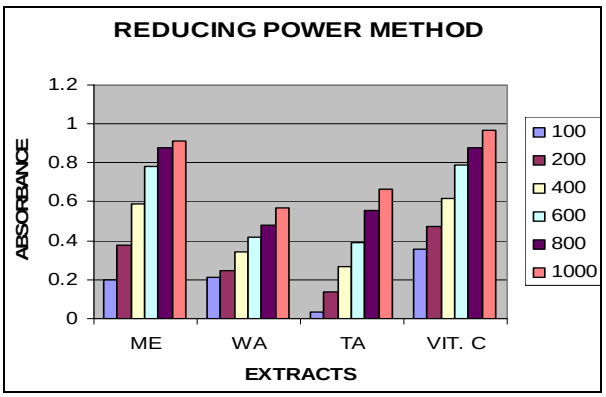

Fig 6: Inhibition by each extract by RP method

For the measurement of the reducing ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of extracts using the method of Oyaizu. The reducing capability of a compound may serve as a significant indicator of its potential antioxidant. Reducing power of the selected diluted extract found to be significant and as good as ascorbic acid (Table no.3)
The antioxidant activity has been reported to be concomitant with development of reducing power. The reducing power of extracts increased with increasing concentration. All the amounts of extracts showed significant activities when compared to control and these differences were statistically significant.
From all different methods of evaluating antioxidant activity it was concluded that methanol, water and total aqueous extract showed activity but methanol showed the most significant one of the all extracts.

Conclusion

Since the review of the plant suggested to claim many ethno-medicinal claims the antioxidant activity on the plant was been carried out by DPPH radical scavenging activity, nitric oxide scavenging method and determination of reducing power. The result reveals that the methanol and aqueous extracts maintains good antioxidant activity.

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