



Comparative Analysis of Phytochemical Screening and Antioxidant Activity of Some Medicinal Plants

M.Suriyavathana^{1*}, V.Sivanarayan²

¹Department of Biochemistry, Periyar University, Salem - 636 011, Tamilnadu, India.

²Department of Biochemistry, Vysya College, Salem - 636 007, Tamilnadu, India.

*Corresponding author's E-mail: sivanarayanbio@gmail.com

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ABSTRACT

Medicinal plants (*Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, *Vitex negundo*) were analyzed for their phytochemical analysis and antioxidant activity. Methanolic extracts of these plants (leaves) showed high antioxidant activity and *Delonix elata* possessed the highest percent inhibition of DPPH, reducing power, nitric oxide scavenging activity and hydroxyl radical scavenging activity amongst all studied plants. The results also revealed the presence of substantial amount of bioactive constituents comprising phenol content (31.7 mg in 0.5 ml of 1:10 g ml⁻¹) and flavonoids (29.4 mg in 0.5 ml of 1:10 g.ml⁻¹) where *Delonix elata* was observed comparatively richer source of these phytochemicals. The results provided the evidence that the studied medicinal plants are to be potent source of natural antioxidant and medicinally important compounds.

Keywords: Medicinal plants, phytochemical analysis, Antioxidant activity.

INTRODUCTION

Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search for some plants with strong antioxidant activity which could serve as good candidates for the development of standardized phytomedicine. Reactive oxygen species (ROS) such as super oxide, hydrogen peroxide, hydroxyl and nitric oxide start attacking the cell proteins, lipids and carbohydrates leading to many physiological diseases. Free radicals are involved the development of degenerative diseases. Free radicals contribute to more than one hundred disorders in human beings including atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS¹. Antioxidants can be effective in preventing free radical formation by scavenging them or increasing their decomposition rate and suppressing such disorders². Currently, there is a growing interest toward natural antioxidants of herbal resources. In present study, we carried out a systematic record of the relative free radical scavenging activity in selected medicinal plants. The seven medicinal plants studied were collected from Kolli hills, Namakkal, Tamil Nadu. The plant materials were cleaned and powdered. The botanical names, family names, English/Common names and parts used are presented in Table 1.

MATERIALS AND METHODS

Plant materials

The leaves of *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, *Vitex negundo* were collected from collected from Kolli hills, Namakkal District.

Preparation of extracts for phytochemical analysis

The plant material(s) was allowed to shadow dry and afterwards pulverized by using mortar and pestle. 10 grams pulverized material were dissolved in 100 ml of solvent (Methanol and water) and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for analysis³.

Phytochemical analysis

Alcoholic extracts were subjected to preliminary phytochemical screening as per the standard methods. This analysis determines the biologically active non-nutritive compounds that contribute to the flavor, color, and other characteristics of plant parts. Examples of these are alkaloids, flavonoids, steroids, tannins, cardiac glycosides, saponin etc.

Test for Carbohydrates

A small quantity of the extract was dissolved separately in 4 ml distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

Molisch's test

Filtrate was treated with 2-3 drops of 1% alcoholic alpha-naphthol solution and 2 ml of Cone. H₂SO₄ was added along the sides of the tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Test for Alkaloids

Mayer's test

1.2 ml of the extract was taken in a test tube, 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent



were added. Formation of yellowish buff colored precipitate confirmed the presence of alkaloid.

Wagner's test

Ten milliliters of the extract was acidified by adding 1.5 % v/v of HCL and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloid.

Test for Phytosterol

The extract was refluxed with solution of alcoholic potassium hydroxide till complete saponification takes place. The mixture was diluted and with ether. The ether layer was evaporated and the residue was tested for presence of phytosterol.

Libermann burchard test

Test residue was dissolved in few drops of diluted acetic acid; 3ml of acetic anhydride was added followed by drops of concentrated sulphuric acid. Appearance of bluish green color shows the presence of phytosterol.

Salkowski test

10mg of the extract was dissolved in 1ml of chloroform; 1ml of concentrated sulphuric acid was added carefully along the sides of test tube. The red color was produced, indicating the presence of sterols

Test for Saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

1 ml of the extract was treated with 1 % lead acetate solution. Formation of white precipitated indicates the presence of saponins.

Test for Proteins and Free Amino acids

Biuret test

Equal volumes of 5 % NaOH solution and 1% copper solution were added to 1.0 ml of the extract. Appearance of pink color shows the presence of protein.

Test for Flavonoids

Shinoda's test

In a test tube containing 0.5 ml of extract, 5 – 10 drops of diluted HCL and small piece of ZnCl or magnesium were added and the solution was boiled for few minutes. In the presence of flavonoids reddish pink color was produced.

Alkaline Reagent Test

To 1.0 ml of the extract, few drops of dilute sodium hydroxide were added. An intense yellow color was produced in the plant extract, which become colorless on addition of a few drops of dilute acid indicate the presence of flavonoids.

Test for Carotenoids

1g of each specimen sample was extracted with 10ml of chloroform in a test tube with vigorous shaking. The resulting mixture was filtered and 85% sulphuric acid was added, a blue color at the interface showed the presence of carotenoids.

Table 1: Brief introduction of medicinal plants Botanical Name used

Botanical Name	English/Common Name	Part	Family	Medicinal use
<i>Delonix elata</i>	Poinciana	Leaf	Fabaceae	Antioxidant, anti-inflammatory
<i>Cassia auriculata</i>	Tanners cassia, Avaram	Leaf	Fabaceae	Antidiabetes, antibacterial
<i>Catharanthus roseus</i>	Madagascar periwinkle	Leaf	Apocynaceae	Antidiabetes, antimalarial
<i>Hibiscus sabdariffa</i>	Rosella fruit	Leaf	Malvaceae	Anticancer
<i>Mukia maderasptna</i>	None recorded	Leaf	Cucurbitaceae	Antioxidant
<i>Ruellia tuberosa</i>	Minnie root	Leaf	Acanthaceae	Anti-inflammatory, Antidiabetic
<i>Vitex negundo</i>	Five-leaved chaste tree	Leaf	Lamiaceae	Antioxidant, anticough

Quantitative determination

Total Flavonoids Estimation

Aluminium chloride colorimetric method was used for flavonoids determination⁴. Each plant extracts (0.5 ml of 1:10 g ml⁻¹) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a UV/Visible spectrophotometer. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg ml⁻¹ in methanol.

Total Phenols Estimation

Total phenols were determined by Folin Ciocalteu reagent⁵. A dilute extract of each plant extract (0.5 ml of 1:10 g ml⁻¹) or tannic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted water) and aqueous sodium carbonate (4 ml, 1M). The mixtures were allowed to stand for 15 min and total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 50, 100, 150, 200, 250 mg l⁻¹ solutions of tannic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of tannic acid equivalent (mg g⁻¹ of dry mass), which is common reference compound.



In vitro antioxidant activity

DPPH free radical scavenging assay

The effect of alcoholic extract on DPPH radical was according to the procedure described by Moure, Franco, two milliliters of 3.6×10^{-5} M methanolic solution of DPPH (sigma) were added to 50 μ l of a methanolic solution (1mg ml⁻¹) of the anti oxidant. The decreased in the absorbed at 515nm was continuously recorded in a spectrophotometer for 16 min at room temperature. The scavenging effect (decreased of absorbance at 515nm) was plotted against the time and percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 min duration.

$$\% \text{ DPPH activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

Determination of reducing power

The reducing power of different solvent extract was determined according to the method of Ozaizu as described by yen et al. The alcoholic extract ranges from (10-50mcg) in 1ml of methanol was mixed with a phosphate buffer (5ml, 0.2M, pH 6.6) and potassium ferric cyanide (5.0ml, 1.0%), the mixture was incubated at 50°C for 20 minutes. A portion 5.0ml of trichloro acetic acid (10%) was added to the mixture, which was then centrifuged at 3600nm for 10 minutes. The upper layer of the solution (5.0ml) was mixed with distilled water (5.0ml) and ferric chloride (1.0ml, 0.1%), and then the absorbance was read spectrophotometrically at 700nm. Higher absorbance of their action mixture indicated greater reducing power.

Nitric oxide generation and assay of Nitric oxide scavenging method

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO. SNP (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (10-50mcg/ml) of the drug dissolved in ethanol and water and incubated at 25°C for 180 minutes. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm and referred to the absorbance of ascorbic Acid, used as a positive control treated in the same way with Griess reagent.

$$\text{Nitric Oxide scavenged (\%)} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

Where, Control = Absorbance of control reaction and test = Absorbance in the presence of the samples of extracts.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from the Fe³⁺/ascorbate/EDTA/H₂O₂ system. The reaction mixture contained deoxyribose (2-8mM), FeCl₃ (0.1mM), EDTA (0.1 mM), ascorbate (0.1 mM), H₂O₂ (1mM), KH₂PO₄-KOH buffer (20mM, pH 7.4) and various concentrations (10-50 mcg/ml) of standard drug in a final volume of 1 ml. The reaction mixture was incubated for 1 hr at 37°C; deoxyribose degradation was measured at 532 nm (Mary et al. 2002).

RESULTS AND DISCUSSION

Table 2 shows the preliminary phytochemical screening of the alcoholic (Methanol) extracts & water extracts of *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, and *Vitex negundo*. The extracts showed the presence and absence of alkaloids, saponins, steroids, flavonoids, phenolic compounds, and proteins. The alcoholic extracts gave more positive results than aqueous extract.

Table 3 shows the amount of phenols & flavonoids present in *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. Among the seven plants, *Delonix Elata* contains notable quantity of Phenols and Flavanoids.

Table 4 shows the Invitro antioxidant activity of alcoholic (Methanol) extracts of *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. The percentage of DPPH (free radicals) scavenging activity is increased with respect to the concentration of the plant extracts. Among the seven plants, *Delonix Elata* shows notable DPPH scavenging activity.

Table 5 shows the reducing power of alcoholic (Methanol) extracts of *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. Higher absorbance of the reaction mixture indicated greater reducing power. Among the seven plants, *Delonix Elata* shows notable reducing power.

Table 6 shows the reducing power of alcoholic (Methanol) extracts of *Cassia auriculata*, *Catharanthus roseus*, *Delonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. The percentage of nitric oxide (free radicals) scavenging activity is increased with respect to the concentration of the plant extracts. Among the seven plants, *Delonix Elata* shows notable Nitric oxide scavenging activity.

Table 7 shows the hydroxyl of alcoholic (Methanol) extracts of *Cassia auriculata*, *catharanthus roseus*, *Ddelonix elata*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. The percentage of



hydroxyl radical (free radicals) scavenging activity is increased with respect to the concentration of the plant

extracts. Among the seven plants, *Delonix Elata* shows notable hydroxy radical scavenging activity.

Table 2: Phytochemical Analysis of alcoholic (Methanol) extract and water extract of seven plants

TEST	<i>Cassia auriculata</i>		<i>Catharanthus roseus</i>		<i>Delonix elata</i>		<i>Hibiscus sabdariffa</i>		<i>Mukia maderasptna</i>		<i>Ruellia tuberosa</i>		<i>Vitex negundo</i>	
	E	W	E	W	E	W	E	W	E	W	E	W	E	W
Extract	E	W	E	W	E	W	E	W	E	W	E	W	E	W
Carbohydrate & Glycosides - Molischs test	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Test for alkaloids														
Mayers reagent	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Wagner reagent	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Test for phytosterol														
Liebermann burchard test	+	-	-	-	+	-	+	-	+	-	+	-	-	+
Salkowski test	+	+	-	+	+	+	+	+	+	+	+	-	+	+
Test for saponins														
Foam test	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Test for protein & free amino acid														
Biuret test	+	-	-	-	-	+	-	-	-	-	-	-	-	+
Test for flavonoids														
Shinoda's test	+	+	-	+	+	+	+	+	+	+	+	+	+	-
Alkaline Reagent Test	+	+	-	-	+	+	-	+	+	+	+	+	+	+
Test for carotenoids														
	-	-	+	-	+	-	+	-	+	-	+	-	-	-

E – Ethanolic extract; W – Aqueous extract; (+) presence; (-) absence

Table 3: Estimation of phenols and flavonoids

Secondary metabolites	Plant Name						
	<i>Cassia auriculata</i>	<i>Catharanthus roseus</i>	<i>Delonix elata</i>	<i>Hibiscus sabdariffa</i>	<i>Mukia maderaspatna</i>	<i>Ruellia tuberosa</i>	<i>Vitex nigundo</i>
PHENOL (mg) (0.5 ml of 1:10 g ml ⁻¹)	30	5.7	31.7	7.2	30	7	8.6
FLAVONOIDS (mg) (0.5 ml of 1:10 g ml ⁻¹)	28	0.6	29.4	24	12	8	26.5

Table 4: DPPH scavenging activity (in %) of Alcoholic (Methanol) extract of seven plants

Con in mcg	Std (Vit C)	<i>Cassia auriculata</i>	<i>Catharanthus Roseus</i>	<i>Delonix elata</i>	<i>Hibiscus sabdariffa</i>	<i>Mukia maderasaptana</i>	<i>Ruellia tuberosa</i>	<i>Vitex negundo</i>
10	74.5	70.7	55	72.2	54.9	75	71.9	28.2
20	82.4	71.3	62.3	80.5	58.1	80	78.4	35
30	88	71.9	69.5	86.8	78.2	81	89.6	54
40	97.3	73.5	75	95.1	80.8	85	89.8	84
50	99.4	75.2	81	97.3	81.4	86	91.1	85

Table 5: Determination of reducing power assay of Alcoholic (Methanol) extract of seven plants

Con in mcg	Std (Vit C)	<i>Cassia auriculata</i>	<i>Catharanthus roseus</i>	<i>Delonix elata</i>	<i>Hibiscus sabdariffa</i>	<i>Mukia maderasptna</i>	<i>Ruellia tuberosa</i>	<i>Vitex negundo</i>
10	0.275	0.220	0.154	0.246	0.196	0.035	0.271	0.100
20	0.621	0.421	0.326	0.429	0.360	0.044	0.441	0.228
30	0.722	0.522	0.589	0.622	0.441	0.064	0.667	0.342
40	0.981	0.698	0.734	0.810	0.598	0.068	0.852	0.467
50	1.221	0.792	0.918	0.941	0.788	0.098	0.999	0.582



Table 6: Nitric oxide scavenging activity (in %) of Alcoholic (Methanol) extract of seven plants

Con in mcg	Std (Vit C)	<i>Cassia Auriculata</i>	<i>Catharanthus roseus</i>	<i>Delonix elata</i>	<i>Hibiscus Sabdariffa</i>	<i>Mukia maderasptna</i>	<i>Ruellia tuberosa</i>	<i>Vitex negundo</i>
10	78.6	64.6	33.2	52.2	68.6	20.5	30.2	35.6
20	83.3	65	45.3	66.2	70.5	25.3	49.2	60.8
30	86.3	74.2	59.1	70.5	71.2	31.7	53	66.9
40	90.2	74.8	63.8	72.5	73.8	37.8	55	68.4
50	91.4	77.7	65.3	73.5	86	47	57	71.7

Table 7: Hydroxyl radical scavenging activity of Alcoholic (Methanol) extract of seven plants

Con in mcg	Std (Vit C)	<i>Cassia auriculata</i>	<i>Catharanthus roseus</i>	<i>Delonix elata</i>	<i>Hibiscus sabdariffa</i>	<i>Mukia maderasptna</i>	<i>Ruellia tuberosa</i>	<i>Vitex negundo</i>
10	40.5	3.8	25.2	37.5	16.6	29.6	14.2	11.5
20	58.4	26.3	38.4	48.1	21.1	40.9	25	31
30	65.4	43.7	40.9	58.1	21.8	48.5	37.2	42.6
40	74.2	55	43.6	64.1	37.2	55.5	46.1	52.6
50	80	66.1	52.1	70.7	40	58.6	54.4	61.5

CONCLUSION

The results revealed the presence of substantial amount of bioactive constituents comprising phenol content (31.7mg in 0.5 ml of 1:10 g ml⁻¹) and flavonoids (29.4mg in 0.5 ml of 1:10 g ml⁻¹) where *Delonix elata* was observed comparatively richer source of these phytochemicals. The results provided the evidence that the studied medicinal plants are to be potent source of natural antioxidant and medicinally important compounds. The results also concluded that the antioxidant activity of alcoholic (Methanol) extracts of *Delonix elata* is higher than that of *Cassia auriculata*, *catharanthus roseus*, *Hibiscus sabdariffa*, *Mukia maderasptna*, *Ruellia tuberosa*, & *Vitex negundo*. The notable quantity of phenols and flavonoids may be responsible for its higher antioxidant activity.

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REFERENCES

- Kumpulainen JT, Salonen JT, Natural Antioxidants and Anticarcinogens in Nutrition, *Health and Disease*, the Royal Society of Chemistry, UK, 1999, 178- 187.
- Halliwell B, The antioxidant paradox. *Lancet*, 355, 2000, 1179-1180.
- Kokate, C.K. 1994. Practical pharmacognosy, Vallabh Prasashan Kakatiya University, Warangal, A.P. India 109-114.
- Chang C, Yang M, Wen H and Chem J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal*, 10, 2002, 178-182.
- McDonald S, Prenzler PD, Autolovich M and Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem.*, 73, 2001, 73-84.
- Moure A., Cruz M.J., Franco D., Dominguez J.M., Sineiro J., Nuniez M.J., Parajo J.C. Natural antioxidants from residual sources. *Food Chem.* 72, 2001, 145-171.
- Oyaizu, M. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. *Japan Journal of Nutrition*. 44, 1986, 307-315.
- Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGB 761. *Biochem Biophys Res Commun*, 15, 1994, 748–755.
- Mary NK, Shylesh BS, Babu BH, Padikkala L. Antioxidant and hypolipidaemic activity of herbal formulation – Liposen. *Indian J Exp Bio*, 140, 2002, 901-904.

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