



## COMPARATIVE PHYTOCHEMICAL AND ANTIOXIDANT STUDIES ON ROOTS OF *STEREOSPERMUM COLAIS* & *STEREOSPERMUM SUAVEOLENS*

S. Latha<sup>1</sup>, S. Seethalakshmi<sup>2</sup>, D.Chamundeeswari<sup>1</sup>, R. Senthamarai<sup>3</sup>, S. Shanthi<sup>1</sup> and X. Fatima Grace<sup>4</sup>

<sup>1</sup>Department of Pharmacognosy, <sup>4</sup>Department of Pharmaceutics, Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai, Tamilnadu, India.

<sup>2</sup>Department of Pharmacology, Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University Porur, Chennai, Tamilnadu, India.

<sup>3</sup>Department of Pharmacognosy, Periyar Pharmaceutical Sciences for Girls, Trichy, Tamilnadu, India.

### ABSTRACT

**Aim:** *Stereospermum colais* (SC) and *Stereospermum suaveolens* (SS) are known as “Patala” has a sound traditional and rational background in Ayurvedic system of Medicine. There is no investigation regarding the antioxidant potential of both the plants. So the aim of this study is to screen and compare the antioxidant effect by *in vitro* methods using various extracts of the roots of *Stereospermum colais* and *Stereospermum suaveolens*. **Materials & Methods:** Successive extracts of the roots of SC and SS were prepared and used for the phytochemical screening and *in vitro* antioxidant study. The antioxidant potential was evaluated using 1,1- Diphenyl -2- picrylhydrazyl (DPPH) and nitric oxide radical scavenging methods. **Results & conclusion:** The roots of SC and SS showed the presence of terpenoids, flavonoids, anthraquinones, glycosides, phenols, tannins, carbohydrates, saponins, proteins and aminoacids. The results revealed that the extracts showed a concentration dependent free radical scavenging activity compared to the standard ascorbic acid and curcumin. The ethanolic extract showed maximum activity among all the extracts in both the methods. The antioxidant potential is higher in SS compared to SC in both the methods.

**Key words:** *Stereospermum colais*, *Stereospermum suaveolens*, Antioxidant.

### INTRODUCTION

In recent years there has been an increased interest in the development of "Natural Antioxidants". Various reports showed that plant derived products play a vital role in scavenging free radicals. Antioxidants protect the human body against free radicals that may cause pathological effects such as ischemia, asthma, anaemia, inflammation, neuro-degeneration, and parkinson's diseases (Narendhirakannan and Smeera, 2010).

Antioxidants protect living organisms from damage due to production of free radicals and associated

lipid peroxidation, protein damage and breaking of DNA strand. Reactive oxygen species such as hydroxyl radicals, superoxide radicals, singlet oxygen and hydrogen peroxide are regularly generated as by-products of biological reaction which plays an important role in cell metabolism including energy production, phagocytosis and intercellular signaling. There are proven results that plant products such as flavonoids, polyphenols, terpenes exerted an antioxidant activity (Asha Poorna et al., 2012).

So a potential antioxidant from a natural source is essential. Hence the present study was designed to explore and compare the antioxidant potential of the two plants *Stereospermum colais* (SC) and *Stereospermum suaveolens* (SS).

Corresponding Author

**S. Latha**

Email: rebekah.latha@gmail.com

*Stereospermum colais* (SC) and *Stereospermum suaveolens* (SS) are large deciduous trees distributed throughout India. The plants are commonly known as 'Pathiri' in Tamil and 'Parral' in Hindi (The Wealth of India, 1959; Varier, 1996). Both the plants are mentioned as "Patala" in Ayurveda. The roots of SC are bitter, diuretic, cardiogenic and anti-inflammatory and febrifuge (Chopra *et al.*, 1956). The roots of SS are useful in inflammations, asthma, fever and affections of brain (Krithikar and Basu, 1999). Phytoconstituents such as  $\beta$ -sitosterol and lapachol were isolated from the roots of these plants (Purushotaman and Natarajan, 1974; Joshi *et al.*, 1977). The roots of SC reported to possess activity against *Salmonella typhosa* and antidiabetic activity (Bhatnagar *et al.*, 1961). So far no antioxidant studies have been carried out using the roots of these plants. An attempt was made to screen and compare the antioxidant potential of *Stereospermum colais* and *Stereospermum suaveolens* as well the presence of phytoconstituents held responsible for the activity.

## MATERIALS AND METHODS

### Plant material

The roots of SC and SS were collected from Madurai, Tamilnadu and Tirupathi, Andhrapradesh respectively. The plant specimens were authenticated by Prof. P. Jayaraman, PARC, Tambaram, Chennai. The voucher specimens [PARC/2007/80 and PARC/2012/1080 respectively] were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Sri Ramachandra University, Chennai.

### Preparation of extracts

The roots of SC and SS were shade dried, powdered into a coarse powder. The powdered plant materials were successively extracted with Pet. Ether (PESC, PESS), Chloroform (CHSC, CHSS), ethylacetate (EASC, EASS) and ethanol (EESC, EESS). The filtered

extracts were concentrated using rotary vacuum evaporator.

### Phytochemical Analysis

Preliminary phytochemical analysis was performed using standard methods (Madhu C Divakar, 2002) for the identification of flavonoids, glycosides, anthraquinones, terpenoids, steroids, phenols, tannins, carbohydrates, saponins, proteins and aminoacids.

### Determination of antioxidant activity

#### DPPH radical scavenging assay (Yohozowa *et al.*, 1998)

Aliquots of 0.1ml of various extracts (10, 50, 100, 200, 400, 800, 1000 $\mu$ g/0.1ml) were mixed with 1.9ml of DPPH solution (200 $\mu$ M in ethanol) and incubated in dark condition for 20min at 37 $^{\circ}$ C. The absorbance of the reaction mixture was recorded at 517nm. Ascorbic acid (AS) was used as standard. The radical scavenging activity was determined using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

#### Nitric oxide radical scavenging Assay (Alderson *et al.*, 2001)

Aliquots of 2ml of sodium nitroprusside (10mM in phosphate buffered saline) were mixed with 1ml of various extracts (10, 50, 100, 200, 400, 800, 1000 $\mu$ g/ml) and incubated for 4 hours at 37 $^{\circ}$ C. To the above solution, 0.5ml of Griess reagent was added and the absorbance was measured at 546nm. Curcumin (CUR) was used as standard. The radical scavenging activity was determined using the formula

$$\text{Percentage inhibition} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

**Table 1. Phytochemical analysis of SC**

Extracts	Terpenoids	Flavanoids	Steroids	Anthroquinones	Glycosides	Carbohydrates	Alkaloids	Quinones	Phenols	Tannins	Saponins	Proteins & amino acids
PESC	-	-	-	-	-	-	-	-	-	-	-	-
CESC	-	-	-	-	-	-	-	-	-	-	-	-
EASC	-	+	-	-	+	+	-	-	+	+	-	+
EESC	-	+	-	-	+	+	-	-	+	+	+	+

Note : (+) Positive, (-) Negative

**Table. 2 – Phytochemical analysis of SS**

Extracts	Terpenoids	Flavanoids	Steroids	Anthroquinones	Glycosides	Carbohydrates	Alkaloids	Quinones	Phenols	Tannins	Saponins	Proteins & amino acids
PESC	+	-	+	+	-	-	-	+	-	-	-	-
CESC	-	-	-	-	+	+	-	-	-	-	-	-
EASC	-	+	-	-	+	-	-	-	+	+	-	+
EESC	-	+	+	+	+	+	-	+	+	+	+	+

Note : (+) Positive, (-) Negative

### DPPH radical scavenging assay

**Table 3.DPPH radical scavenging activity of SC**

Concentration ( $\mu\text{g}/0.1\text{ml}$ )	Percentage inhibition				
	AS	PESC	CHSC	EASC	EESC
10	21.92 $\pm$ 4.01	0.24 $\pm$ 0.44	12.86 $\pm$ 3.27	23.74 $\pm$ 1.16	29.31 $\pm$ 1.89
50	19.41 $\pm$ 3.76	0.53 $\pm$ 4.51	18.54 $\pm$ 1.42	26.15 $\pm$ 0.42	27.35 $\pm$ 0.83
100	62.09 $\pm$ 3.79	1.02 $\pm$ 2.65	22.46 $\pm$ 3.71	33.49 $\pm$ 6.7	38.83 $\pm$ 5.64
200	74.19 $\pm$ 3.88	2.02 $\pm$ 0.43	28.73 $\pm$ 8.47	27.40 $\pm$ 5.2	60.61 $\pm$ 1.30
400	81.96 $\pm$ 3.65	3.28 $\pm$ 4.98	30.15 $\pm$ 5.56	32.80 $\pm$ 1.9	68.29 $\pm$ 0.11
800	86.14 $\pm$ 3.02	8.16 $\pm$ 3.20	36.85 $\pm$ 5.12	44.18 $\pm$ 3.60	73.30 $\pm$ 1.06
1000	91.76 $\pm$ 2.13	15.70 $\pm$ 3.50	43.52 $\pm$ 0.99	49.54 $\pm$ 1.57	78.65 $\pm$ 1.40

Values are expressed as mean $\pm$ S.D of three experiments

**Table 4.DPPH radical scavenging activity of SS**

Concentration ( $\mu\text{g}/0.1\text{ml}$ )	Percentage inhibition				
	AS	PESS	CHSS	EASS	EESS
10	21.92 $\pm$ 4.01	1.72 $\pm$ 0.44	9.41 $\pm$ 1.27	8.45 $\pm$ 2.89	18.25 $\pm$ 1.47
50	19.41 $\pm$ 3.76	3.90 $\pm$ 4.51	10.82 $\pm$ 1.42	14.50 $\pm$ 0.83	37.56 $\pm$ 7.08
100	62.09 $\pm$ 3.79	6.10 $\pm$ 2.65	15.01 $\pm$ 3.71	29.69 $\pm$ 5.64	52.18 $\pm$ 3.88
200	74.19 $\pm$ 3.88	11.01 $\pm$ 0.43	18.27 $\pm$ 2.47	33.34 $\pm$ 1.30	64.37 $\pm$ 2.94
400	81.96 $\pm$ 3.65	14.93 $\pm$ 4.98	29.67 $\pm$ 5.56	53.73 $\pm$ 0.11	76.18 $\pm$ 2.42
800	86.14 $\pm$ 3.02	18.82 $\pm$ 3.20	38.35 $\pm$ 5.12	62.85 $\pm$ 1.06	87.99 $\pm$ 1.17
1000	91.76 $\pm$ 2.13	23.32 $\pm$ 2.13	48.85 $\pm$ 0.99	76.54 $\pm$ 1.40	91.17 $\pm$ 1.01

Values are expressed as mean $\pm$ S.D of three experiments

### Nitric oxide radical scavenging Assay

**Table 5. Nitric oxide Radical Scavenging Activity of SC**

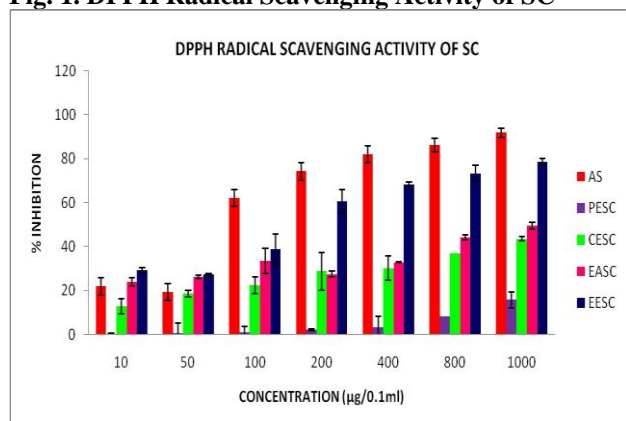
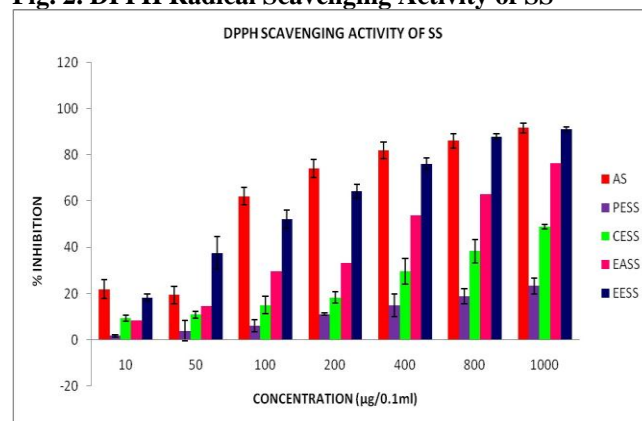
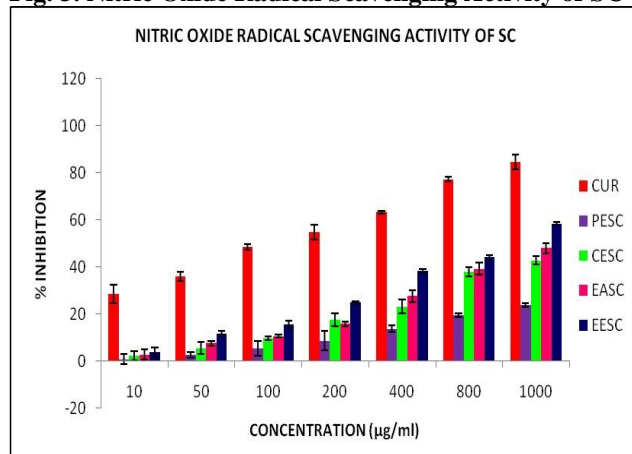
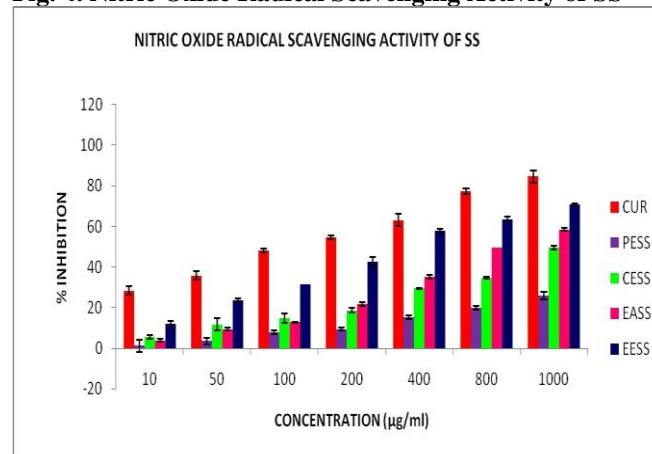
Concentration ( $\mu\text{g}/\text{ml}$ )	Percentage inhibition				
	CUR	PESC	CHSC	EASC	EESC
10	28.47 $\pm$ 3.82	0.59 $\pm$ 2.14	2.13 $\pm$ 1.92	2.70 $\pm$ 2.2	3.69 $\pm$ 1.87
50	35.84 $\pm$ 2.08	2.49 $\pm$ 1.12	5.29 $\pm$ 2.54	7.45 $\pm$ 0.93	11.70 $\pm$ 1.06
100	48.29 $\pm$ 1.18	5.30 $\pm$ 3.08	9.42 $\pm$ 0.82	10.56 $\pm$ 0.49	15.42 $\pm$ 1.54
200	54.72 $\pm$ 3.09	8.48 $\pm$ 4.1	17.45 $\pm$ 2.82	15.73 $\pm$ 0.99	24.76 $\pm$ 0.45
400	63.19 $\pm$ 0.68	13.59 $\pm$ 1.29	23.11 $\pm$ 3.02	27.56 $\pm$ 2.56	38.27 $\pm$ 0.65
800	77.25 $\pm$ 0.84	19.45 $\pm$ 0.69	37.82 $\pm$ 1.84	39.15 $\pm$ 2.47	43.95 $\pm$ 0.92
1000	84.54 $\pm$ 3.21	23.69 $\pm$ 0.79	42.68 $\pm$ 1.73	47.89 $\pm$ 2.05	58.42 $\pm$ 0.67

Values are expressed as mean $\pm$ S.D of three experiments

**Table 6. Nitric Oxide Radical Scavenging Activity of SS**

Concentration ( $\mu\text{g/ml}$ )	Percentage inhibition				
	CUR	PESS	CHSS	EASS	EESS
10	28.47 $\pm$ 1.9	1.24 $\pm$ 2.83	5.81 $\pm$ 0.94	3.89 $\pm$ 0.67	11.93 $\pm$ 1.56
50	35.84 $\pm$ 2.05	3.59 $\pm$ 1.78	11.78 $\pm$ 3.02	9.41 $\pm$ 0.78	23.72 $\pm$ 1.05
100	48.29 $\pm$ 0.93	7.92 $\pm$ 0.86	14.93 $\pm$ 2.29	12.81 $\pm$ 0.19	31.62 $\pm$ 0.04
200	54.72 $\pm$ 0.99	9.37 $\pm$ 0.74	18.67 $\pm$ 1.05	21.78 $\pm$ 1.12	42.69 $\pm$ 2.47
400	63.19 $\pm$ 3.02	15.49 $\pm$ 0.87	29.62 $\pm$ 0.26	35.24 $\pm$ 1.04	57.94 $\pm$ 0.95
800	77.25 $\pm$ 1.38	19.99 $\pm$ 0.89	34.78 $\pm$ 0.49	49.69 $\pm$ 0.06	63.62 $\pm$ 1.05
1000	84.54 $\pm$ 3.06	25.93 $\pm$ 1.84	49.67 $\pm$ 0.91	58.52 $\pm$ 0.69	70.84 $\pm$ 0.34

Values are expressed as mean $\pm$ S.D of three experiments

**Fig. 1. DPPH Radical Scavenging Activity of SC****Fig. 2. DPPH Radical Scavenging Activity of SS****Fig. 3. Nitric Oxide Radical Scavenging Activity of SC****Fig. 4. Nitric Oxide Radical Scavenging Activity of SS**

## RESULTS

### Phytochemical analysis

The roots of SC showed the presence of flavonoids, glycosides, phenols, tannins, carbohydrates, saponins, proteins and aminoacids are shown in Table.1. The roots of SS showed the presence of flavonoids, glycosides, terpenoids, steroids, phenols, anthraquinones, tannins, carbohydrates, saponins, proteins and aminoacids are shown in Table.2.

### *In vitro* antioxidant activity

The extracts of SC and SS were screened for its antioxidant potential using various concentrations by *in vitro* models. The DPPH assay results were presented in table-3&4 and fig.1&2. The results of nitric oxide radical scavenging assay were given in table-5&6 and fig.3&4.

## DISCUSSION & CONCLUSION

The roots of SC showed the presence of flavonoids, glycosides, phenols, tannins, and SS showed

the presence of flavonoids, glycosides, terpenoids, steroids, phenols, anthraquinones, tannins. The results revealed that all the extracts showed a concentration dependent free radical scavenging activity compared to the standard ascorbic acid and curcumin. The ethanolic extract of SC and SS showed maximum activity among all the extracts in both the methods. Several studies have been proved that flavonoids, phenols and tannins are compounds rich in antioxidant potential. The presence of these compounds in ethanolic extract may be accountable for the maximum activity compared with other extracts.

The antioxidant potential was higher in SS compared to SC in both the methods. The presence of terpenoids, steroids and anthraquinones in SS may attribute for the efficacy. Further studies need to be carried out for the quantification of secondary metabolites which is responsible for the antioxidant activity.

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