IN VITRO ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF METHANOL EXTRACT OF *STEREOSPERMUM COLAIS* (BUCH.- HAM. EX.DILLW).

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ABSTRACT:

Background and Objectives: *Stereospermum colais (Bignoniaceae) has good ethnopharmacological value in Ayurvedic system of Medicine. However, its antioxidant and anti-inflammatory effects are not explored yet. Therefore, the objective of this study is to evaluate the antioxidant and anti-inflammatory effect of the methanol extract of the leaves of Stereospermum colais by in vitro methods.*

Methods : *The antioxidant activity was studied using 1,1- Diphenyl -2- picrylhydrazyl (DPPH) and nitric oxide radical scavenging activity. In vitro anti-inflammatory activity was evaluated using membrane stabilization assay.*

Results : *The methanol extract of the leaves of S. colais showed the presence of carbohydrates, saponins, flavonoids, glycosides, terpenoids, phenols, anthraquinones, tannins, proteins and aminoacids. In the in vitro antioxidant assays, the free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in both the models which is comparable to that of the standard curcumin. In the in vitro anti inflammatory assay the methanol extract showed significant anti-inflammatory activity at the concentration of 1000µg/ml which is comparable to that of the standard drug Diclofenac sodium.*

Conclusion : *The methanol extract of the leaves of Stereospermum colais showed significant antioxidant and anti-inflammatory effect. The results obtained in the present study indicate that Stereospermum colais is a potential source of natural antioxidant and anti-inflammatory agent.*

Key words: *Stereospermum colais, Antioxidant, Anti-inflammatory.*

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INTRODUCTION:

In recent years much attention has been devoted to natural antioxidant and their association with health benefits.[1] It is commonly accepted that the reactive oxygen species play an important role related to many chronic and degenerative diseases such as aging[2], cancer, coronary heart disease, Diabetes mellitus, atherosclerosis, neurodegenerative disorders[3] and inflammation.[4]

Inflammation is a complex localized response to foreign substances. In many inflammatory disorders there is excessive activation of phagocytes, production of O_2, $OH^{\cdot}$ radicals as well as non-free radical species [H_2O_2].[5] These free radicals are the main culprits in lipid peroxidation resulting in membrane destruction followed by production of mediators and chemotactic factors.[6] Hence the agents that can scavenge these reactive oxygen species can be beneficial in the treatment of inflammatory disorders.

Various medicinal plants provide relief from symptoms comparable to that obtained from allopathic medicines.[7] It has been suggested that many anti-inflammatory drugs may exert some of their effects by scavenging oxidants, and decreasing formation of Reactive Oxygen Species (ROS) by activated phagocytes.[8]

Stereospermum colais (Buch.- Ham. Ex Dillw) Mabberley (Family: Bignoniaceae) is a large deciduous tree distributed throughout India.[9] It is commonly known as 'Pathiri' in Tamil and 'Parral' in Hindi. In Ayurveda, the leaves of the plant are used in otalgia, odontalgia, rheumatalgia, malarial fever and wounds.[10] So far its antioxidant and anti inflammatory properties have not yet been pharmacologically evaluated. Hence, the present study was undertaken to evaluate the antioxidant and anti-inflammatory activity of *Stereospermum colais* by *in vitro* methods.

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MATERIALS AND METHODS

Plant material

The leaves of *Stereospermum colais* was collected from Alagar kovil hills, Madurai, Tamilnadu and authenticated by Prof. P. Jayaraman, PARC, Tambaram, Chennai. The voucher specimen [PARC/2007/80] was deposited at the Department of Pharmacognosy, Madras Medical College, Chennai.

Preparation of plant extract

The leaves of *Stereospermum colais* was shade dried, powdered and defatted using petroleum ether (60-80^{0}C) and successively extracted with methanol. Extract was filtered through vacuum filter and the filtrate was concentrated in vacuum evaporator.

Preliminary Phytochemical Analysis

Preliminary phytochemical analysis[11] was carried out for carbohydrates, saponins, flavonoids, glycosides, terpenoids, steroids, tannins, proteins and aminoacids.

Determination of antioxidant activity

DPPH radical scavenging assay

DPPH radical scavenging activity was done using the method of Yohozowa *et al*.[12] The reaction mixture containing 1ml of DPPH solution (200μM in ethanol) with different concentrations of the extract was shaken and incubated for 20min at room temperature. The resultant absorbance was recorded at 517nm. The percentage inhibition was calculated 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

The nitric oxide radical scavenging activity was done using the method of Alderson et al.[13] 3ml of reaction mixture containing sodium nitroprusside (10mM in phosphate buffered saline) and various concentrations of the extracts were incubated at 37^{0}C for 4 hours. To the incubation solution, 0.5ml of Griess reagent was added and the absorbance was read at 546nm. The percentage inhibition was calculated using the formula

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

***In vitro* anti-inflammatory activity**

Membrane stabilization assay

The HRBC membrane stabilization method has been used to study the anti-inflammatory activity.[14] Blood was collected from the healthy volunteers and mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000rpm and packed cells were washed with isosaline (0.85%, PH 7.2) and a suspension was made with isosaline (10% v/v).

The assay mixture contained 1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%), 0.5ml of HRBC suspension and 1ml of various concentrations of the extract. Diclofenac sodium was used as reference drug. In the control solution instead of hyposaline, 2ml of distilled water was added. The mixtures were incubated at 37^{0}C for 30min and centrifuged. The absorbance of the supernatant solution was read at 560nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization was calculated using the formula.

$$\text{Percentage membrane stabilization} = 100 - \frac{\text{O.D of drug treated sample}}{\text{O.D of control}} \times 100$$

RESULTS

Preliminary phytochemical analysis

The methanol extract of the leaves of *S. colais* showed the presence of carbohydrates, flavonoids, glycosides, terpenoids, phenols, anthraquinones, tannins, proteins and aminoacids are shown in Table.1. Flavonoids, phenols and tannins are compounds basically have been proven with antioxidant activity. So the presence of these compounds may be responsible for the activity.

Table. 1 – Preliminary phytochemical analysis

S.No.	Tests	Methanol extract
1.	Carbohydrates	+
2.	Saponins	-
3.	Flavonoids	+
4.	Glycosides	+
5.	Terpenoids	+
6.	Steroids	-
7.	Tannins	+
8.	Phenols	+
9.	Anthraquinones	-
10.	Alkaloids	-
11.	Proteins and aminoacids	+

Note : + Positive, - Negative

***In vitro* antioxidant activity**

Several concentrations ranging from 62.5μg/ml to 2000μg/ml of the methanol extract of *S. colais* were tested

Table.2 – *In vitro* antioxidant activity of *S. colais*

S. No.	Concentration (μg/ml)	Percentage inibition			
		DPPH Assay		Nitricoxide scavenging Assay	
		Methanol Extract	Curcumin	Methanol Extract	Curcumin
1.	62.5	23.66 $\pm$ 0.70	48.35 $\pm$ 0.80	16.43 $\pm$ 0.77	30.47 $\pm$ 0.83
2.	125	40.67 $\pm$ 1.09	52.27 $\pm$ 0.89	22.25 $\pm$ 0.79	45.82 $\pm$ 0.90
3.	250	59.46 $\pm$ 0.95	64.62 $\pm$ 0.86	31.63 $\pm$ 0.81	59.19 $\pm$ 0.55
4.	500	65.53 $\pm$ 0.81	79.63 $\pm$ 1.17	42.48 $\pm$ 0.74	67.35 $\pm$ 0.54
5.	1000	74.68 $\pm$ 1.17	89.33 $\pm$ 0.82	58.27 $\pm$ 1.06	75.20 $\pm$ 0.43
6.	2000	82.73 $\pm$ 0.92	92.47 $\pm$ 1.30	70.50 $\pm$ 1.21	87.63 $\pm$ 0.54

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

for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test compounds in a concentration dependent manner upto the given concentration in both the models. Table 2 reveals the reductive capability of the methanol extract compared to that of the standard curcumin.

***In vitro* anti-inflammatory activity**

HRBC membrane are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity. It was observed from the Table.3, that the methanol extract shows significant anti-inflammatory activity at the concentration of 1000μg/ml which is comparable to that of the standard drug diclofenac sodium (200μg/ml). The anti inflammatory activity of the extract was concentration dependent.

Table.3 – *In vitro* anti-inflammatory effect of *S. colais*

S. No.	Concentration (μg/ml)	Percentage protection	
		Methanol extract	Standard (Diclofenac Sodium)
1.	10	08.13 ± 0.27	-
2.	50	12.43 ± 0.20	-
3.	100	19.31 ± 0.33	49.32 ± 0.18
4.	200	24.88 ± 0.26	78.15 ± 0.35
5.	400	31.63 ± 0.62	-
6.	800	53.09 ± 0.13	-
7.	1000	72.66 ± 0.56	-

Values are expressed as mean ± S.D of three experiments

DISCUSSION

Free radical oxidative stress has been implicated in the pathology of a wide variety of clinical disorders. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals inhibiting lipid peroxidation and by many other mechanisms and thus prevent disease.

DPPH is a relatively stable free radical, the assay is based on the measurement of the scavenging activity of antioxidants towards the stable DPPH. From the present study it may be postulated that *S. colais* reduces the radical to the corresponding hydrazine when it reacts with hydrogen donors in the antioxidant principles.[15]

Nitric oxide (NO) is a very unstable species, under aerobic condition it reacts with O_2 to produce stable product nitrate & nitrite through intermediates NO_2, N_2O_4 & N_3O_4. In the present study, the nitrite produced by the reaction mixture was reduced by the methanol extract of *S. colais*. This may be due to the antioxidant principles in the extract which compete with oxygen to react with nitric oxide.[16]

The extract exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane[17] and its stabilization implies that the extract may as well stabilize lysosomal membrane. From the above study it was concluded that the methanol extract of *S. colais* has significant antioxidant and anti inflammatory activity.

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