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**ESTIMATION OF TOTAL PHENOLIC CONTENTS, TOTAL FLAVONOID CONTENTS AND MUSCLE CO-ORDINATION ACTIVITY OF ETHANOLIC EXTRACT OF *STEREOSPERMUM SUAVEOLENS* DC**

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**ABSTRACT**

The ethanolic extract of *Stereospermum suaveolens* (ESS) was carried out with the help of Soxhlet apparatus. The total phenolic contents of extract was determined by Folin-Ciocalteu method using spectrophotometer and flavonoid contents was measured by aluminium chloride colorimetric assay. The muscle co-ordination potential was determined by using rotarod apparatus and digital actophotometer in mice. The total phenolic contents (TPC) in the extract using the standard calibration curve of gallic acid was found to be 38.5mgGAE/g and that of total flavonoid contents (TFC) by using quercetin was quantified 26.1 mg QE/g of extract. Similarly, ethanolic extract 200 and 400 mg/kg showed significant dose dependent muscle coordination activity using rotarod apparatus and actophotometer. Treatment with extract at a dose of 400 mg/kg showed maximum significant ( $p < 0.001$ ) decreased fall off time (101.2 s) as compared to control. Further, extract at a dose 400 mg/kg showed maximum 19.14% and 69.77% reduction in the locomotor activity after 30 and 60 min of administration when compared with control group using actophotometer. Thus, the ethanolic extract of *Stereospermum suaveolens* contains high extent of phenolics and flavonoids and has significant centrally acting skeletal muscle relaxant activity.

**KEYWORDS**

Flavonoids, Muscle coordination, Phenolics and *Stereospermum suaveolens*.

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**INTRODUCTION**

Traditional Indian system of medicine reported several plants and their therapeutic usage due to presence of active metabolites. The plants continue to play pivotal role in searching modern medicine, and today more than one quarter of drugs are either derived from plants or contain plant extracts<sup>1</sup>. A folk medicinal plant *Stereospermum suaveolens*

(Roxb) DC. Is a large deciduous tree commonly known as 'Patala' and belongs to family Bignoniaceae that is found throughout the most parts of India<sup>2</sup>. Traditionally, a decoction of the root is used for the treatment of inflammation, pain, asthma and in the preparation of Ayurvedic formulation known as 'Dashmula'<sup>3</sup>; while the flowers mixed with honey were given orally for the control of hiccups<sup>4</sup>. Ethno medicinally, stem bark is used as diuretic and liver tonic<sup>5</sup>. A literature review on *Stereospermum suaveolens* plant reported that, modern researcher proved the plant for its potential antihyperglycemic and antioxidant activities, hepatoprotective activity, anticancer activity, and antihyperlipidemic Activity<sup>6</sup>. Moreover, previous phytochemical studies showed the presence of lapachol, dehydro- $\alpha$ -lapachone, stereokunthol B, and stereoehenols A and B in the bark, and stereolensin, scutellarein, 6-hydroxy luteolin, dinatin, and dinatin-7-glucuroniside in the leaves<sup>7</sup>.

Similarly, anxiety and musculoskeletal disorders are extremely dramatic and debilitating disorders which are impossible to offer effective treatment strategies for the patients. Over the past decades, there has been exhaustive study of a variety of neurobiological aspects of anxiety. Currently, benzodiazepines are most widely prescribed drugs in anxiety and musculoskeletal disorders, but use is limited due to their unwanted side effects<sup>8</sup>. Various herbal remedies are present that possess lesser side effects than the conventional drugs and thus are safer to use.

Since, crude plant drugs form the basis for the manufacture of numerous medicinal preparations, accurate determination of drug identity, therapeutically active constituent, isolation and pharmacological screening techniques are extremely important to emphasize the way to discover new drugs. Thus, the objective of present study was to quantify total phenolic and flavonoid content; and muscle co-ordination activity of ethanolic extract of plant *Stereospermum suaveolens* DC by using rotarod apparatus and actophotometer.

## MATERIAL AND METHODS

### Chemicals and reagents

Gallic acid (Mallinckrodt Chemical Inc., USA), Folin-Ciocalteu reagent (Sigma Aldrich, USA), Quercetin, Diazepam (Lupin Laboratories Ltd., India). All the reagents and solvents were of analytical grade and are prepared freshly before the experimentation.

### Plant material and preparation of extract

The fresh dried stem barks were collected from Kolhapur District (Dhorle and Sons Ayurveda) of Maharashtra, India. The plant herbarium was identified and authenticated by Dr. (Mrs.) Anuradha Upadhye, Scientist, Agharkar Research Institute Pune, India and the specimen (Auth.15-117) was deposited in Department of Biodiversity and Palaeobiology.

The shade dried fruits were grounded to fine powder (sieve no 40) and subjected to ethanolic extraction by using continuous Soxhlet apparatus method. The extract was then filtered through Whatman (No-1) filter paper, dried on a rotary evaporator at 45°C under reduced pressure and preserved in airtight containers until further use.

### Animals

Swiss albino mice of either sex (25-30 g) were used for the muscle co-ordination activity. All the experimental animals were maintained under standard husbandry conditions (Temp. 22-28°C; relative humidity 65±10%) and are given standard food pellet (Hindustan Lever) and water *ad libitum* throughout the experimental period. The experimental protocol received approval from the Institutional Animal Ethical Committee (IAEC Clearance: MCPL/IAEC/12-13/04).

### Estimation of total phenolic contents

The estimation of total phenolic contents in plant extract was determined with Folin-Ciocalteu method using spectrophotometer<sup>9,10</sup>. Ethanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture consists of 1 ml of extract and 9 ml of distilled water in a 25 ml volumetric flask. 1 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken vigorously. After 5 minutes, 10

ml of 7% sodium carbonate solution was treated to the mixture. The volume was adjusted up to 25 ml. Similarly, a set of standard solutions of Gallic acid (20, 40, 60, 80 and 100 µg/ml) were prepared in similar manner. Absorbance of test and standard solutions were determined against the reagent blank at 550 nm with UV-Visible spectrophotometer (Shimadzu 1800) after the incubation period of 90 min at room temperature. The total phenolic content was expressed as mg of Gallic acid equivalent (GAE) per g of extract. The absorbance of test sample was performed in triplicate.

#### **Estimation of total flavonoid content**

Total flavonoid content was measured by the aluminium chloride colorimetric assay<sup>11,12</sup>. Briefly, the reaction mixture contains 1 ml of extract and 4 ml of distilled water in a 10 ml volumetric flask. Add, 0.30 ml of 5% sodium nitrite and after 5 minutes, 0.3 ml of 10% aluminium chloride was mixed. After 5 minutes, 2 ml of 1M sodium hydroxide was treated and diluted to 10 ml with distilled water. A similar set of reference standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) were prepared. The absorbance for test and standard solutions were determined against the reagent blank at 510 nm with an UV-Visible spectrophotometer. The total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of extract. The absorbance of test sample was performed in triplicate.

### **MUSCLE CO-ORDINATION ACTIVITY**

#### **Treatment Protocol**

The effective dose of ethanolic extract of *Stereospermum suaveolens* (ESS) 200 and 400 mg/kg, p.o., was selected based on previous published research article<sup>13</sup>. Mice of either sex were allotted randomly into three groups (n=6). Group I served as control which received normal saline (10 ml/kg), group II received standard reference drug Diazepam (4mg/kg, i.p.)<sup>14</sup>. Group III and IV received the ESS orally at a dose of 200 and 400 mg/kg respectively.

To understand exact site of action of extract, to the separate group of animals, the dose of ESS 200 and

400 mg/kg was injected directly into the thigh muscles. The control group received distilled water intramuscularly.

#### **Rotarod apparatus**

The skeletal muscle co-ordination activity of ESS was performed according to method described earlier by Dunham and Miya (1956)<sup>15,16</sup>, using Rotarod apparatus (Carewell, India). Thirty min after i.p and sixty min after oral administration of standard and test extract, mice were kept on rotating rod at a speed of 25 rpm for 5 min cutoff time period. The fall off time from the rotating rod was noted for each mouse. The difference in the fall off time from the rotating rod between the control and treated animals was taken as an index of muscle relaxation.

#### **Actophotometer**

The spontaneous locomotor activity was evaluated with the help of a digital actophotometer (Esal, India)<sup>17,18</sup>. Each animal was placed individually in actophotometer and locomotor activity score of all the animals were recorded after 30 and 60 of standard drug and test extract treatment. The digital counts, as the number of line crossings by animal due to beam interruptions, were recorded for 5 min. The counts resemble to locomotor activity. The percentage reduction in locomotor activity was determined.

#### **Central Vs Peripheral muscle co-ordination property**

To elucidate primary site of action of ESS, the animals were subjected to Actophotometer before and after the treatment of ESS 200 and 400 mg/kg intramuscularly. The locomotor score was counted and percentage reduction activity was determined<sup>18</sup>.

#### **Statistical Analysis**

The values are expressed as mean ± SEM. Statistical analysis was performed with One-way analysis of Variance (ANOVA), followed by Dunnett's test (n=6). The value with p<0.05 was considered to be statistically significant.

### **RESULTS AND DISCUSSION**

Plant materials may contain a range of chemically diverse phenolics and flavonoids. Based on the

strong evidence of biological activities of phenolic and flavonoid compounds, the present investigation has been carried out to determine the total phenolic contents and total flavonoid content present in ethanol solvent extract of *Stereospermum suaveolens* DC and screening for skeletal muscle relaxant activity by using animal models.

#### **Estimation of total phenolic and flavonoid contents**

The total phenolic and flavonoid contents in plant extract depends on the type of solvent used. The solubility of phenols and flavonoids in polar solvents provides high concentration<sup>19,20</sup>. The total phenolic contents in the plant extract using the Folin-Ciocalteu's reagent was expressed in terms of gallic acid equivalent (the standard curve equation:  $y = 0.046x + 0.0476$ ,  $R^2 = 0.9949$ ) (Figure No.1). The calibration curve showed linearity for gallic acid in the range of 20 - 100 $\mu$ g/ml. The values obtained for the concentration of total phenols are expressed as mg of GA/g of extract. The total phenolic contents in the ethanolic extract using the calibration curve, was found to be 38.5mgGAE/g (Table No.1).

Similarly, the concentration of flavonoids in plant extract was determined using colourimetric method with aluminum chloride. The total content of flavonoids was expressed in terms of quercetin equivalent (the standard curve with a correlation coefficient ( $R^2$ ) = 0.9949), mg of QE/g of extract (Figure No.2). In the result, the quantitative analysis of TFC in an extract was found to be 26.1mgQE/g (Table No.1).

#### **Muscle co-ordination activity**

Rotarod is widely used screening method for muscle relaxation and actophotometer for evaluating the locomotor activity in rodents. The results of rotarod study showed that the ethanolic extract of *Stereospermum suaveolens* possess a dose dependent skeletal muscle relaxant activity in experimental animals (Figure No.3). Treatment with extract at a dose of 400 mg/kg showed highest significant ( $p < 0.001$ ) decreased fall off time (101.2 s) and as that of standard Diazepam showed significant ( $p < 0.001$ ) (89.51 s) decreased fall off

time, and sliding time and increase climbing time (motor co-ordination) when compared with the control group.

Similarly, in the present study, ESS produced significant dose dependent depressant effect in mice using actophotometer as compared to control. The percentage of reduction in the locomotor activity with dose 200mg/kg of ESS after 30 and 60min was found to be 9.40% and 58.53% respectively. Besides, ESS at dose 400mg/kg showed maximum 19.14% and 69.77% reduction in the locomotor activity after 30 and 60min of administration when compared with control group (Table No.2). The reference standard drug diazepam showed significant 49.99% and 2.36% decreased locomotor activity after 30 and 60min of administration.

However, in second experiment, where the extract of ESS was given directly into the skeletal muscle, there was no statistically significant ( $p > 0.05$ ) decrease in the locomotor activity at dose 200 as well as 400 mg/kg when compared with control (Table No.3). This indicated that, crude extract may not have peripheral action.

The present study showed a dose-dependent increase in muscle relaxation and decrease in locomotion with two different doses of ESS implies depression effect on the central nervous system. It has been well-known that an augment in the concentration of inhibitory neurotransmitter, gamma-amino butyric acid (GABA) may lead to CNS depressant effect<sup>21</sup>. The earlier investigation showed that plant containing flavonoids, phenols and tannins were found to be ligands for the GABA receptors in the central nervous system<sup>22</sup>. The observed muscle relaxant effect of ESS may be due to the agonistic effect on GABA/benzodiazepine receptor complex via membrane hyper polarization which leads to a decrease in the firing rate of critical neurons in the brain. Therefore, muscle co-ordination potential of ESS may be attributed to presence of high contents of phenols and flavonoids. In addition, results of Table No.2 and 3 proved that, ESS may have centrally acting muscle co-ordination property.

**Table No.1: Estimation of TPC and TFC in ethanolic extract of *Stereospermum suaveolens***

S.No	Estimations	Concentration
1	Total phenolic contents(mg GAE/g)	38.5±0.65
2	Total flavonoid contents (mg QE/g)	26.1±0.33

Each value is the mean of three analyses ± SEM

**Table No.2: Effect of ESS on locomotor activity by using Actophotometer**

S.No	Treatment	Number of Movements			% Reduction Activity	
		Before Treatment	After 30 min. Treatment	After 60 min. Treatment	After 30min	After 60min
1	Control (Normal saline 10 ml/kg)	196.52 ± 1.23	197.05 ± 1.12	195.89 ± 1.98	-	-
2	Diazepam (4 mg/kg)	197.83 ± 1.81	98.56 ± 1.11**	34.56 ± 1047***	49.99%	82.36%
3	ESS (200 mg/kg)	201.14 ± 1.23	178.53 ± 1.96*	81.24 ± 1.36**	9.40%	58.53%
4	ESS (400 mg/kg)	198.01 ± 1.45	159.34 ± 1.58**	59.23 ± 1.51***	19.14%	69.77%

Values are Mean ± SEM, One way analysis of Variance followed by multiple Dunnett t-test (n=6), \*p<0.05,

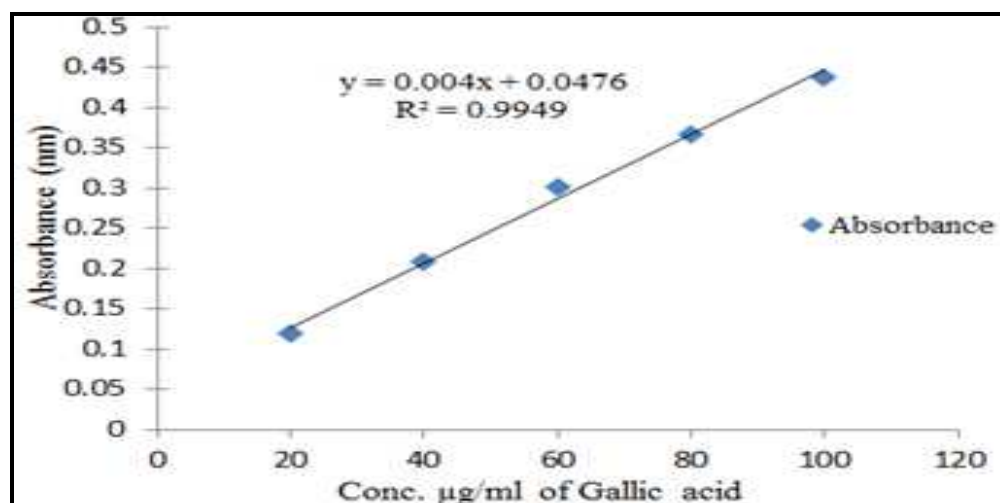
\*\*p<0.01, \*\*\*p<0.001 as compared with control

**Table No.3: Effect of ESS on locomotor activity after intramuscular injection**

S.No	Treatment	Number of Movements			% Reduction Activity	
		Before Treatment	After 30 min. Treatment	After 60 min. Treatment	After 30 min	After 60 min
1	Control (Normal saline 10 ml/kg, i.m)	188.72±1.63	187.15±1.42	185.99±1.71	-	-
2	ESS (200mg/kg, i.m)	187.13±1.53	184.53±1.86	179.14±1.38	1.40%	3.69%
3	ESS (400mg/kg, i.m)	155.11±1.35	181.84±1.78	174.23±1.81	2.86%	6.33%

Values are Mean ± SEM, One way analysis of Variance followed by multiple Dunnett t-test (n=6), \*p<0.05,

\*\*p<0.01, \*\*\*p<0.001 as compared with control



**Figure No.1: Linear calibration curve of standard gallic acid**

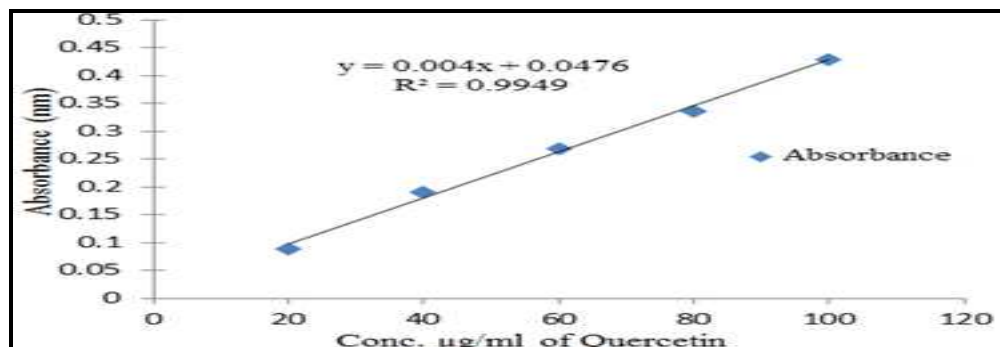


Figure No.2: Linear calibration curve of standard quercetin

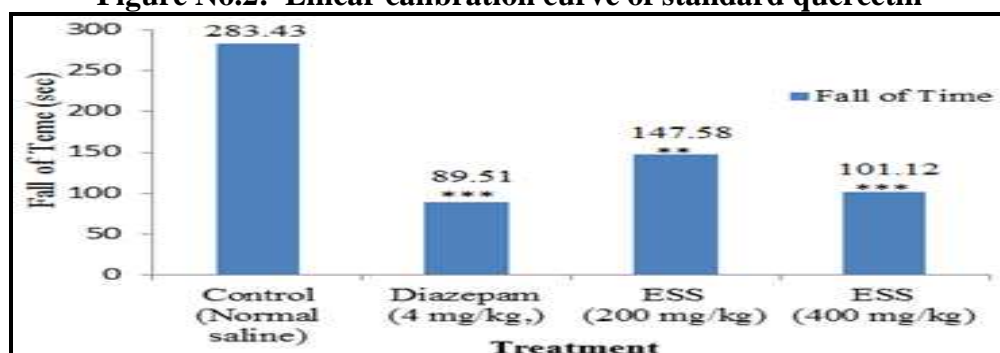


Figure No.3: Effect of ESS on Muscle co-ordination activity. Values are Mean  $\pm$  SEM, One way analysis of Variance followed by multiple Dunnett t-test ( $n=6$ ), \* $p<0.05$ , \*\* $p<0.01$ , \*\*\* $p<0.001$  as compared with control

## CONCLUSION

The Phytochemicals study have been acknowledged increasing attention because of interesting new discoveries considering their biological activities especially polyphenols and flavonoids. The present study showed that, ethanolic extract of plant *Stereospermum suaveolens* showed high contents of phenolics and flavonoids and having skeletal muscle relaxant potential. Further, detailed investigation and isolation of bioactive metabolites is necessary to understand therapeutic usefulness of plant.

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## CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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