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STUDIES ON THE EVALUATION OF ANXIOLYTIC ACTIVITY OF ETHANOLIC BARK EXTRACT OF *Symplocos racemosa* roxb. IN SWISS ALBINO MICE: A PRELIMINARY EXPERIMENTAL STUDY

N. AMANI[1*], V HIMABINDHU[1], K RAVEENDRA BABU[2], ANNAM HEMANTH KUMAR[2] AND M. CHINNA ESWARIAH[3]

1: Assistant Professor, Anurag Pharmacy College, Ananthagiri, Suryapeta, Telangana-508206, India

2: Assistant Professor, Anurag Pharmacy College, Ananthagiri, Suryapeta, Telangana-508206, India

3: Principal and Professor, Anurag Pharmacy College, Ananthagiri, Suryapeta, Telangana-508206, India

***Corresponding Author: N. Amani: E Mail: amaninukabathini@gmail.com**

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1. INTRODUCTION

A displeased situation of internal disorder which is known as anxiety. The mental states of fear, worry, and unease are called anxiety. Numerous conditions can cause anxiety, such as panic disorders, generalized anxiety disorder, specific phobias, social anxiety disorder, separation anxiety disorder, agoraphobia, and others. The bark extract of *Symplocos racemosa* (Lodhra) was used in this investigation. This bark has expectorant, astringent, anti-inflammatory activity. Studies have revealed that it is also useful in eye diseases bleeding gums, asthma, skin diseases, haemorrhages etc. Since many years it is being used safely to treat many GI tract infections. According to Ayurveda this Lodhra reduces fever and cures bleeding gums. A wide range of bioactive constituents including flavonoids, tannins, loutrine, linoleic acid symplocososide, symploveroside etc. have been isolated from this plant **[1-5]**.

2. MATERIALS AND METHODS

2.1 Chemicals

Each chemical employed in the investigation is of analytical quality. Working standards of Diazepam (SD Fine Chemicals Ltd., Mumbai) 3mg/kg and phenobarbitol of 20mg/kg were used. 70% of ethanol (Merck, India) is used as a solvent.

2.2 Plant material collection and extraction

The plant material of *Symplocos racemosa (*Lodhra) was obtained from the nearby market and verified by the botany department of Sri Venkateshwara University, Tirupathi, under the direction of Dr. K. Madhava Chetty. The bark pieces were gathered, ground using a grinder to a coarse powder, and then allowed to dry in the sun. A thimble or porous bag made of sturdy filter paper was filled with finely ground pure medication and placed inside the chamber. After being heated in the flask, the extraction solvent (ethanol) condensed in the condenser. The condensed extract falls into the thimble that holds the raw medication. The liquid in the siphon chamber moves into the flask when the liquid level reaches the top of the siphon tube. This procedure is repeated until not a single drop of solvent from the siphon tube evaporates without leaving any trace. The primary benefit of this is that a lot of medication is extracted using a lot less solvent.

2.3 Evaporation of the solvent:

The obtained extracts were evaporated by distilling the solvent and letting it evaporate at low temperatures until it became dry.70% ethanol has been used as the solvent in this investigation. Ethanol has been shown in studies to evaporate at a temperature of 172°F, or 78°C. There was a semisolid concentration of the finished product. For the purpose of drying, the product is kept in an oven. It yielded a powdery substance. After weighing them, the percentages of the various extractive values were computed using the plant material's air-dried weight.

Percentage yield value of Ethanolic bark extract of *Symplocos racemosa* plant was calculated using the formula:

% Yield **[12]** of ethanolic extract= (Weight of extract) / (Powder taken for extraction) × 100

2.4 Preliminary phytochemical Screening:

Alkaloids, carbohydrates, tannins, saponins, steroids, phenols, and flavanoids were all analyzed as part of the initial phytochemical screening of Symplocos racemosa bark extract using conventional procedures **[9-12]**.

2.5 Acute toxicity studies:

The animal research was approved under approval number 1629/PO/a/12/CPCSEA, and it was conducted in accordance with CPCSEA guidelines. Prior to animal testing, the extracts' acute oral toxicity was assessed

in accordance with Organization for Economic Cooperation and Development (OECD) norms number 423. For the investigation, Swiss Albino mice weighing 20–30g were given a single dosage of 2000 mg/kg of extract. Over the course of 14 days, the treated animals were observed to record general clinical signs and symptoms, as well as mortality. There are four groups of animals, each including five animals. Until the end of the trial, no deaths were reported, indicating that the 2000 mg/kg dose was safe. For further testing, dosages of 1/5, 1/10, and 1/20 milligrams per kilogram, or 100 mg and 200 mg, respectively, were selected. The greatest dose was utilized to ascertain the fatal dose, or LD50, which is the dosage at which 50% of the test group's animals died.

2.5 *In vivo* studies:

Swiss albino mice weighing about 20-30 gm were used for the study. Proper veterinary care and daily observation of animals ware maintained in order to perform the study. Usually, animals were quarantined for atlaest 7 days in order to adapt for the environmental conditions and room temperatures. These animals were kept in cages with proper bedding material. Normal temperature should be maintained between 18-29°C. During the experiment, a relative humidity approximately 55% should be maintained. Four groups of six animals each were assigned at random to the animals. Vehicle control is in Group I. As test subjects, Groups II and III were given oral doses of plant extracts (100 mg/kg and 250 mg/kg, respectively) of *Symplocos racemosa*. Group IV:Positive control treatment with diazepam, 3 mg/kg i.p which. Following an hour of care, the animals were examined for several research tests.

2.6 Elevated plus maze

The Elevated Plus Maze (EPM) was utilized to observe animal behaviour related to anxiety. The apparatus majorly consists of two open arms with (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 10 cm) that enter from a common central platform (5 cm × 5 cm). The whole EPM was raised 50 centimeters above the ground.

Following extract treatment, each mouse was placed in an open arm position on the EPM center platform, and it was watched for five minutes. Stopwatches were used to record the number of times each animal entered the open and closed arms as well as the duration of each animal's presence in each setting (conventional parameters). Following each mouse's recording, alcohol was used to wipe the platform and the inside faces of the closed arms. Following the dosage, the animals' entrances into closed and open arms were watched. Following the recording of the entries, the animals were placed in a CO2 (carbon dioxide) chamber and put to death.

2.7 Stress Induced hyperthermia (SIH) test:

Ten animals each cage, six of which were marked. One by one, in a precise order, the mice were taken out of the cage and given different treatments: phenobarbitol (20 mg/kg, i.p.) for the positive control group, a vehicle for the negative control group, and two doses of plant extracts for the test groups. One minute intervals were used to treat each animal in a particular cage in turn. The mice were taken out of the cage one minute at a time after 60 minutes, and their rectal temperature was taken. Based on the observation that the mice removed later had a greater body temperature than the animals removed earlier in the same cage, this experiment was conducted. SIH (ΔT°) was defined as the variation between the first three mice's mean temperature and the final three mice's mean temperature.

2.8 Statistical Analysis

The findings were reported as mean ± S.E.M. One-way analysis of variance (ANOVA) was used for statistical analysis. If the overall P- value was considered statistically significant ($P< 0.05$).

3. RESULTS

3.1 Extraction results:

The ethanolic extract yield value from *Symplocos Racemosa* was found to be 14%.

3.2 Phytochemical screening:

The results of the hydroalcoholic extract of *Symplocos racemosa* were positive for carbohydrates, alkaloids, glycosides, tannins, and saponins, but negative for steroids and turpinoids was illustrated in **Table 1**.

3.3 Effect of anxiolytic related like behaviour of *Symplocos Racemosa* plant extract in EPM test:

The mice were exposed to EPM in order to determine how the *Symplocos Racemosa* plant extract affected their behavior correlated to anxiety. At a dosage of 250 mg/kg, the *Symplocos Racemosa* therapy in EPM substantially increased the number of entrances into open arms from 0.0 in the control group to 1.5. Diazepam increased the percentage of entry and the amount of time spent in open arms in the positive control group. *Symplocos Racemosa* similarly caused a decrease in the percentage of entry and the amount of time spent in closed arms **(Figure 1)**.

To understand the effect of *Symplocos racemosa* plant extract in anxiolytic-related behaviour the mice were subjected to SIH test. In Stress induced hyperthermia test, phenobarbitol reduced the temperature from 37.2°C to 26.5°C. In SIH, like phenobarbitol, *Symplocos racemosa* strongly reduced ΔT° from 38.2°C to 27.5°C at the dose of 250 mg/kg. In addition *Symplocos Racemosa* also rduced ΔT° from 38.3°C to 28.7°C at the dose of 100 mg/kg **(Figure 2)**.

Table 1: Preliminary phytochemical screening of methanolic extract of *S. saman* leaves

1	Test for carbohydrates Molish test Fehling test Benedict test Barfoed test	 + + + +	6	Test for steroids and terpenoids Liebermann-burchard test Salkowski test	 - -
2	Test for proteins and amino acids Million test Biuret test Ninhydrin test	 - - -	7	Test for tannins and phenolic compounds Ferric chloride test Lead acetate test Bromine water	 + + +
3	Test for fixed oils and fats Saponification test	 -	8	Test for flavonoids Shinoda test Alkaline reagent test	 - -
4	Test for alkaloids Mayers test Wagners test Dragendroff test Hagers test	 + + + +	9	Test for saponin glycosides Foam test Haemolysis test	 + +
5	Test for glycosides Kellar killani test Borntragers test	 + -			

Table 2: The effect of *Symplocos Racemosa* bark extract in both open arm entries, closed arm entries, total arm entries and ratio open arm entries/ total arm entries (OE/TE) to closed arms entries/ total arm entries (CE/TE) in EPM test

Parameters	water	*Symplocos racemosa (100mg/kg p.o)*	*Symplocos racemosa* (250mg/kg p.o)	Diazepam 3mg/kg in i.p
Open arms entries	0.0±0.0	1.1±0.4	1.5±0.39	1.49±0.5
Closed arms entries	0.0±0.0	4.0±1.7	3.9±1.6	2.2±0.7
Total arms entries	0.0±0.0	45±7	35±7	30±5
Ratio of OE/TE vs CE/TE	0.0±0.0	64±23	62±28	87±23

Data are mean. S.E.M, n = 6, $p < 0.01$, $p < 0.001$, ANOVA followed by Dunnett (HND). Diaz = Diazepam in EPM test

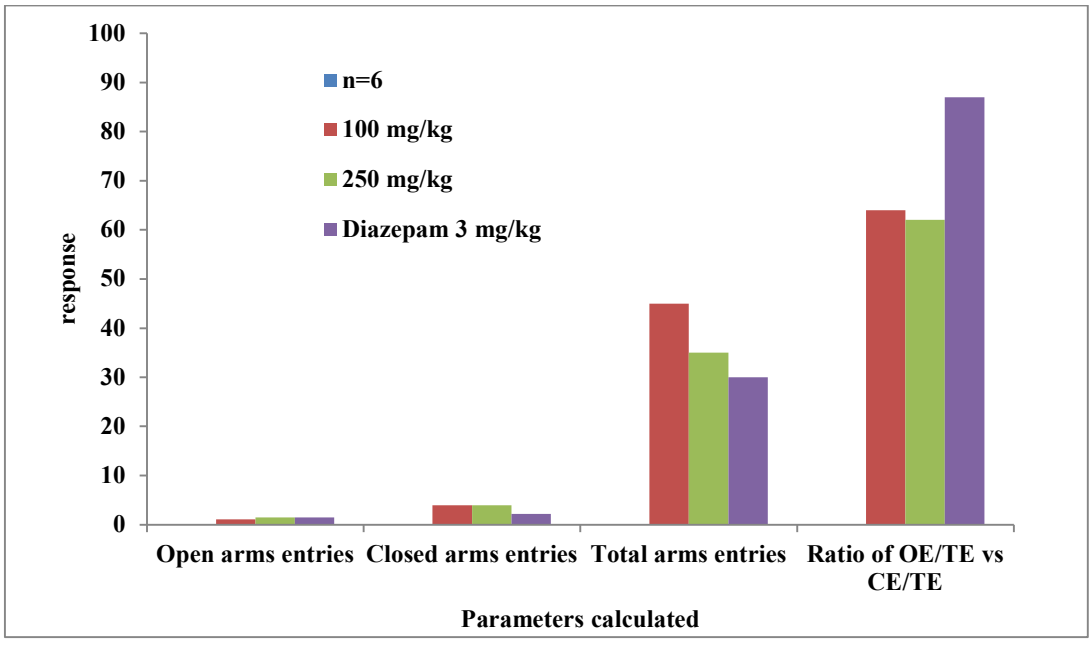

Figure 2: The above figure illustrates the effect of *Symplocos racemosa* bark extract in open arm entries, closed arm entries, total arms entries and ratio open arms entries/ total arms entries (OE/TE) to closed arm entries/ total arm entries (CE/TE) in EPM test and body temperature in SIH test

Table 3: Effect of *Symplocos Racemosa* bark extract in SIH test

Parameters	Before administration	After administration of Phenobarbitol (20mg/kg)
Water	37.6±1.1	30.5±0.6
Symplocos Racemosa (100mg/kg)	38.3±1.0	28.7±0.6
Symplocos Racemosa (250 mg/kg)	38.2±0.8	27.5±0.4
Phenobarbitol (20mg/kg)	37.2±1.6	26.5±0.9

Data are mean. S.E.M, n = 6 or 10, p < 0.01, p < 0.001, ANOVA followed by Dunnett (HND). Diaz = Diazepam in EPM test, PHO = Phenobarbitol in SIH test.

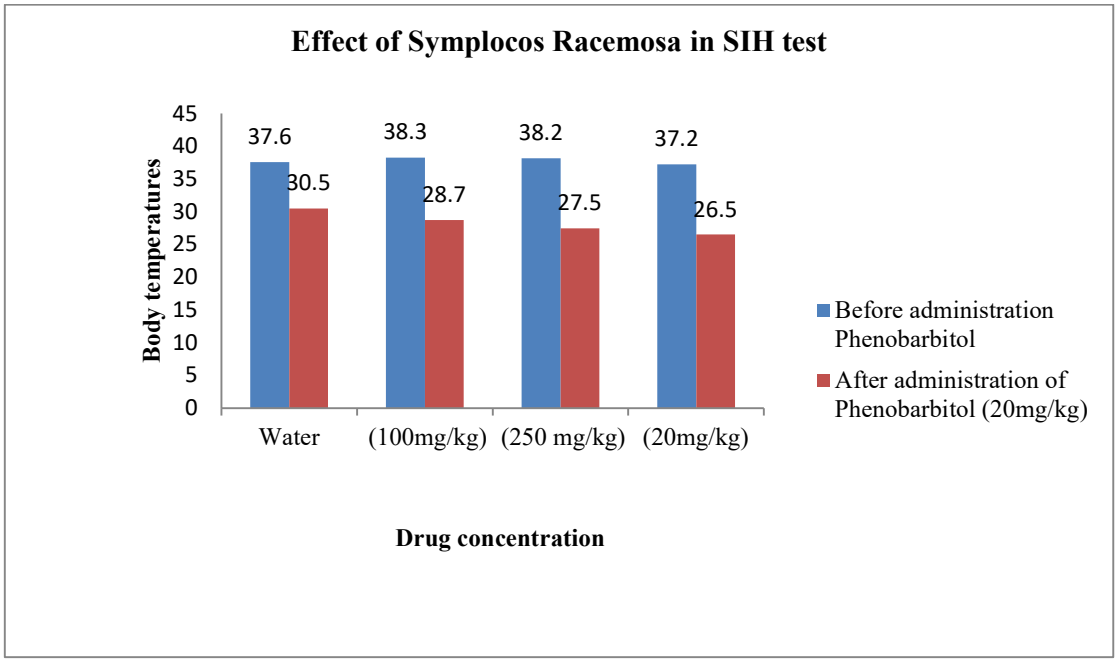

Figure 3: The above figure shows the effect of *Symplocos Racemosa* bark extract in SIH test

4. DISCUSSION

The results from the above phytochemical studies showed the positive results for alkaloids, carbohydrates and glycosides, saponins, proteins and amino acids. The studies showed the negative results for phytosterols and fixed oils and fats **[4]**.

Acute toxicity tests of the extracts were performed as per OECD guidelines (Organization for Economic Co-operation and Development) under guidelines 423.Swiss albino mice were given 2000mg/kg dose was found to be safe for the experimental study. Treated animals were examined for 24 hours for clinical signs and mortality. No mortality was observed and hence the 2000mg/kg dose was found to be safe for the experimental study **[5-8]**.

5.CONCLUSIONS

The two plants *Symplocos racemosa* enhanced the number of entries into open arms, the percentage of entries, and the amount of time in open arms in the EPM test, demonstrating the anxiolytic activity. In addition, *Symplocos racemosa* reduced the number of entries, percentage of entries and time in closed arms. The correlation of increase in time spent in open arms supported the anxiolytic like activity of the selected plants. The extracts of *Symplocos*

racemosa antagonized the hyperthermia in mice. In SIH test it also reduced the temperatures in both the test samples.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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