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PHYTOCHEMICAL SCREENING STUDY AND CONSTIPATION ACTIVITY OF *SPERMACOCE HISPIDA* L. (RUBIACEAE) BY USING ALBINO RATS

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ABSTRACT: The whole plant of *Spermacoce hispida* L is reported to have great medicinal value. It is found effective systemically in treating, headache, constipation, anti-hypertensive, anti-fertility, Appetite, Scabies, Irritant bowel syndrome and skin disease etc. Intake of 2 to 4 g of the seeds along with a mixture of dry ginger, coriander seed and finger millet reduces the unwanted cholesterol namely LDL and HDL from the body. Traditionally roots, leaves and stem of the *Spermacoce hispida* L are used as anti-eczema, antibacterial and cardio-vascular disorder, etc. Recently it is found that this herb contains Calcium and Phosphorus in the abundance hence administration of this drug in form of chooranam or kudineer (decoction) is recommended in conditions like bone diseases, fractures etc The present investigation was therefore undertaken to evaluate the requisite phytochemical screening and the constipation activity of *Spermacoce hispida* by using albino Wistar rats. Phytochemical analysis was carried out with different chemical reagents. Constipation activity of *Spermacoce hispida* was analyzed by using albino Wistar rats. To cure the irritant bowel syndrome using the whole plant of hydro alcoholic extract instead of loperamide. These studies provided crucial information about the constipation activity of the whole plant hydro alcoholic extract. Further studies may be carried out to characterize and screen the valuable compounds present in the whole plant.

INTRODUCTION: Medicinal plants have been used, since times immemorial in virtually all cultures as a source of medicine. Herbal remedies and plant-based healthcare preparations obtained from traditionally used plants have been traced to the occurrence of natural products with medicinal properties.

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Moreover medicinal plants and herbal remedies are re-emerging medical aids whose contribution and significance in the maintenance of good health and well-being is widely accepted [1].

In addition to the use in the developing world, herbal medicine is used in industrialized nations by alternative medicine practitioners such as naturopathy. A 1998 survey of herbalists in the UK found that many of the herbs recommended by them were used traditionally but had not been evaluated in clinical trials [2]. Many of these herbs have been used traditionally and are rated by herbalists as being safe and effective, but lack evidence to support this from controlled clinical

trials [3]. In Australia, a 2007 survey found that these Western herbalists tend to prescribe liquid herbal combinations of herbs rather than tablets of single herbs [4].

Spermacoce hispida (Rubiaceae) was popularly known as "Nattaiccuri" in Tamil or "Shaggy button weed" in English. The seed-extract of the plant has been used as a remedy for the treatment of many ailments such as internal injuries of nerves and kidney. It is suggested that it removes signs of old age, purify blood and improve vitality and have been used by the tribal's living in the forest regions in the Western Ghats of Kerala since ancient times [5].

It has been also reported that *Spermacoce hispida* is an effective natural drug for the treatment of hypertension [6]. The whole plant is used for medicinal properties; it is widely distributed in the Western Ghats of Kerala and in Maruthamalai forest, which is an extension of the Western Ghats in Tamilnadu [7]. *Spermacoce hispida* was one of the five plants, which contained the maximum amount of flavonoids among 25 plants analyzed [8]. It has been reported that the methanolic extract of this whole plant extract exhibited strong antioxidant activity [9].

At present no known scientific study reported in available literature sources that has been carried out so far an irritable bowel syndrome and constipation activity of the whole plant extract. Therefore, the study is aimed at exploring the plant (whole) *Spermacoce hispida* (Rubiaceae)

MATERIALS AND METHODS:

MATERIAL:

a) Drugs and chemicals: Loperamide Hydrochloride and Carmine were used for this study. All other chemicals and reagents used were of analytical grade.

b) Experimental Animals: Albino Wistar rats of weighing between 150-160gm were used for this study. The study was carried in accordance with the rules and regulations laid by the Institutional Animal Ethics Committee. The animals were housed under standard conditions and room temperature ($25\pm2^{o}C$). All animals were fed with standard rat pelleted diet (M/S Pranav Agro Industries Ltd., India). Under the trade name Amrut rat/mice feed and had free access to tap water ad libitum. The study has got approval from the Institutional Animal Ethics Committee, Animal house facility (Registration No: 743/abc/ CPC SEA), PRIST University, Thanjavur, Tamil Nadu, India.

c) Collection and Authentication of Plant: The plant *Spermacoce hispidae* was collected from the fields in Rustumbada, Narsapuram Mandal of West Godavari District, Andhra Pradesh and used in this study. The plant material was authenticated by Dr. A.B.S. Murthy, M. Sc, Ph. D (Botany) Principal of Sri Y.N College (Autonomous), Narasapuram, West Godavari District, Andhra Pradesh.

METHODS:

Phytochemical Screening: The dried plant powder of *Spermacoce hispida* was subjected to a systematic phytochemical screening by successively extracting with various organic solvents and the extracts were subjected for phytochemical investigation by Qualitative chemical identification tests. Primary metabolites like carbohydrates, proteins, fixed oils, fats, gums and mucilages were analyzed for their presence as per the standard procedures. Likewise the secondary metabolites of alkaloids, flavonoids, saponins, glycosides were also performed in the formulation.

1. **Preparation of extracts:** Fresh plants of *Spermacoce hispida* were collected, cut into small pieces and dried under shade at room temperature for forty five days. The dried plant parts were ground to a fine powder and passed through a sieve. This powder was used for the preparation of different solvent extracts. The powdered sample was subjected to successive solvent extraction taking from non-polar to polar solvents like petroleum ether, benzene, chloroform, ethyl acetate, methanol, hydro alcohol and water. 50gm of the sample was subjected to Soxhlet extraction for 8 hrs with 500ml of the various solvents.

Then, the excessive solvents were removed by using Rotary vacuum evaporator (MAC Buchi type). These extracts were stored in desiccators for further analysis.

2. **Procedure for Phytochemical Screening:** Phytochemical examinations were carried out for all the extracts as per the standard methods.

i. **Detection of Alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

A. **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

B. **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). The formation of brown/reddish precipitate indicates the presence of alkaloids.

C. **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of a red precipitate indicates the presence of alkaloids.

D. **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of a yellow colored precipitate.

ii. **Detection of Carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

A. **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution and add slowly Conc. sulphuric acid in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

B. **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

C. **Fehling's Test:** Filtrates were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of a red precipitate indicates the presence of reducing sugars.

3. Detection of Glycosides: Extracts were hydrolyzed with dil. HCl, and then subjected to test for glycosides.

i. **Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

ii. **Legal's Test**: Extracts were treated with sodium nitroprusside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

4. **Detection of Saponins:**

i. **Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

ii. **Foam Test:** 0.5 gm of the extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. **Detection of Phytosterols:**

i. **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

ii. **Liebermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with a few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. The formation of brown ring at the junction indicates the presence of phytosterols.

6. **Detection of Phenols:**

i. **Ferric Chloride Test:** Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

7. Detection of Tannins:

i. **Gelatin Test:** To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

8. Detection of Flavonoids:

i. **Alkaline Reagent Test**: Extracts were treated with a few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on the addition of dilute acid, indicates the presence of flavonoids.

ii. **Lead acetate Test:** Extracts were treated with a few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

9. Detection of Proteins:

i. **Xanthoproteic Test:** The extracts were treated with a few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

ii. **Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

10. Detection of Diterpenes: Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Pharmacological screening (evaluation of constipation activity):

Experimental Design [10]: A total of 30 animals were used for this study. They were grouped into five groups. Each group comprising of six animals (n=6).

- **Group I:** (Control) received normal saline alone.
- **Group II:** (Constipated control) was treated with the Loperamide hydrochloride (3 mg/kg body weight, p.o).
- **Group III:** Administered orally with 50mg/kg body weight, Hydroalcoholic extract of *Spermacoce hispida* (HESH)
- **Group IV:** Administered orally with 100mg/kg body weight, Hydroalcoholic extract of *Spermacoce hispida* (HESH) **Group V:** Administered orally with 200mg/kg body weight, Hydroalcoholic extract of *Spermacoce hispida* (HESH)

RESULTS AND DISCUSSION:

Observation of Fecal Pellets: The excreted fecal pellets of individual rats were collected daily at the particular time (9.00 am).

The following parameters were determined throughout the duration of the study

- Daily Feed intake
- Daily water intake
- Total number of fecal pellets
- Weight of fecal pellets
- Water content of the pellets

Statistical analysis: Data were expressed as Mean±SD. One way analysis of variance (ANOVA) followed by Bonferroni's Multiple Comparison test was performed to determine significant differences in all parameters.

Values were considered statistically significant at $P < 0.05$ and the report mentioned in **Table 1.**

TABLE 1: STATISTICAL ANALYSIS OF CONSTIPATION ACTIVITY IN HESE

Parameters	Control	Constipated control	HESE 50mg/kg	HESE 100mg/kg	HESE 200mg/kg
Feed intake	21.41±1.052	18.33±0.724#	21.86±0.888	21.98±1.014	21.96±0.605
Water intake	53.27±0.806	42.55±1.920#	54.64±1.503	53.68±0.819	54.61±1.280
Number of fecal pellets	71.00±3.266	42.86±4.947#	62. 86±3.185*	52.86±2.340*	43.00±5.000*
Water content of fecal pellets	1.937±0.116	1.257±0.067#	1.710±0.133*	1.470±0.0503*	1.306±0.054*
Weight of fecal pellets	8.300±0.351	4.414±0.146#	7.257±0.113*	6.357±0.415*	5.243±0.151*

Significant difference from normal group. ($p<0.05$), n=6; *Significant difference from standard treated groups ($p<0.05$), n=6

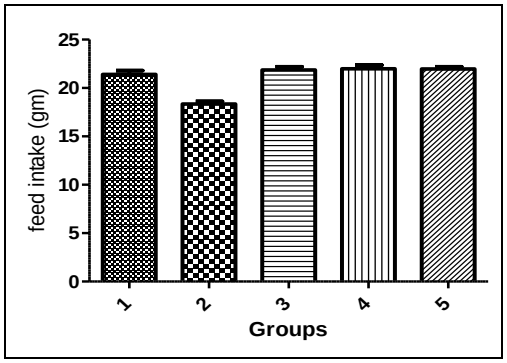

FIG. 1: FEED INTAKE

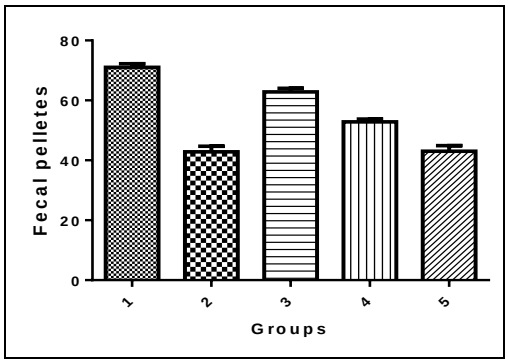

FIG. 2: FECAL PELLETS

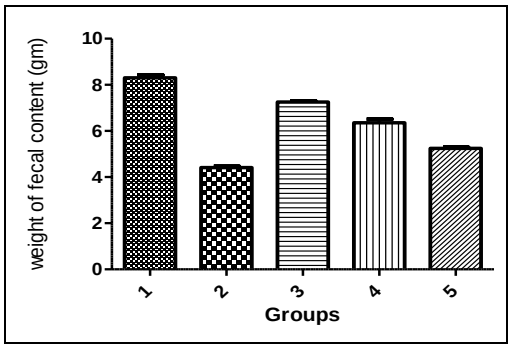

FIG. 3: WEIGHT OF FECAL CONTENT

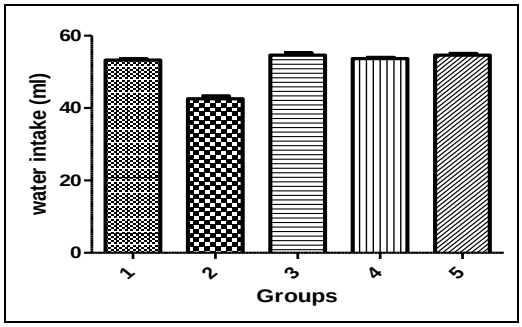

FIG. 4: WATER INTAKE

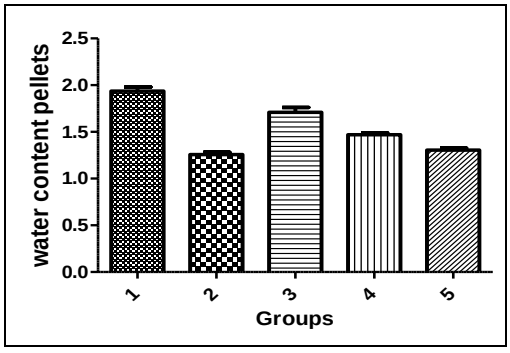

FIG. 5: WEIGHT CONTENT PELLETS

CONCLUSION: The preliminary phytochemical test answered positively for Alkaloids, Carbohydrates, Glycosides, proteins, amino acids, Phytosterols, Triterpenoids, Phenolic compounds and Flavonoids, negative for Saponins, Fixed oils, Fats, gums and mucilages in hydroalcoholic extract.

The hydro alcoholic extract of the whole plant showed significant differences from standard treated groups ($P<0.05$) in constipation activity, when compared to the before treatment at the dose of 200mg/kg was found to be satisfactory.

It can be observed that the constipation activities are comparable to the standard drug of loperamide**.**

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