ijlpr 2023; doi 10.22376/ijlpr.2023.13.5.P6-P13

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Phytochemical Screening and Acute Oral Toxicity Studies of Seeds of *Hyptis Suaveolens* and Fruits of *Coccinia Grandis.*

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Abstract: *Hyptis suaveolens,* a member of the Lamiaceae family, is traditionally used as a carminative and an expectorant. It is also found to have anti-inflammatory, antimicrobial, and anti-cancer potential. *Coccinia grandis,* a member of the Cucurbitaceae family, is found to be anti-helminthic, antioxidant, anti-ulcer, anti-malarial, anti-inflammatory, antipyretic, analgesic, antifungal, antitussive and hypoglycemic in different animal models. The objective of the present study is intended to focus on the phytochemical evaluation of *Hyptis suaveolens* seeds and *Coccinia grandis* fruits and to study their toxic effects by acute oral toxicity study and establish the safety category of these extracts as per organization for economic cooperation and development (OECD-TG-423) guidelines and GHS classification system. *Hyptis suaveolens* seeds, and *Coccinia grandis* fruits were collected, and the extraction was made and stored for phytochemical analysis. The extract was tested for phytochemical constituents like alkaloids, phenol, flavonoids, carbohydrates, triterpenes, sterols, saponins, and tannins. In an acute oral toxicity study, the hydroalcoholic extracts of both plants were administered orally at a dose of 2000mg/Kg b.w. to the three rats in each group in Step I of the individual group. They were observed for 48 hrs and followed for 14 days (step II). They were examined for sensory, physiological, and behavioral parameters and signs of toxicity and mortality. The phytochemical study revealed the presence of many pharmacologically active constituents, and the acute oral toxicity study showed no changes in physical, sensory, physiological, or behavioral parameters, with all animals remaining healthy and active. There were no signs of mortality or moribund status in both step I and II. The results indicate that the hydroalcoholic extracts of Hyptis suaveolens seeds and Coccinia grandis fruits fall under category '5' or 'unclassified' of the GHS system, allowing them to be used safely in pharmaceutical formulations for therapeutic use.

Keywords: OECD, GHS classification system (Globally Harmonised System), *Hyptis suaveolens, Coccinia grandis,* Phytochemical, acute toxicity.

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Received On 19 January, 2023

Revised On 5 April, 2023

Accepted On 18 April, 2023

Published On 1 September, 2023

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Jagannath Narumalla, Dr.D. Sheela and Dr.Rohit Dixit , Phytochemical Screening and Acute Oral Toxicity Studies of Seeds of Hyptis Suaveolens and Fruits of Coccinia Grandis..(2023).Int. J. Life Sci. Pharma Res.13(5), P6-P13 http://dx.doi.org/10.22376/ijlpr.2023.13.5.P6-P13

1. INTRODUCTION

The use of plants as a source of medicine used for treating certain diseases dates back more than 5000 years. According to the World Health Organization, 65-80% of the population uses medicinal plants.[1] Even though the use of the plants has shown promising therapeutic potential, using these plants with the proper scientific support of safety and efficacy can be useful and potentially dangerous. In addition, these medicinal plants may cause serious toxicity for humans on misuse.[2] Traditional use of plant-based medicine for in vivo studies has received great attention during the past few decades.[3] But safety continues to be a major concern with the use of medicinal plants. Therefore, conducting toxicity studies of any medicinal plant extract intended to be used in animals to assess their safety profile is important. Toxicology is a branch of pharmacology that deals with the undesirable effect of phytocompounds on living organisms before being used as a drug or chemical in clinical use.[4] According to OECD guidelines, toxicological studies are very significant in animals like mice, rats, guinea pigs, dogs, rabbits, and monkeys to determine the protection and effectiveness of a new drug. The outcome of toxicological studies gives information on whether or not a new drug must be adopted for clinical use. No drug is permitted to use clinically without clinical trial and toxicity studies according to OECD guidelines 401, 423, and 425.[5] *Hyptis suaveolens*, an annual, perennial herb that grows in subtropical, tropical, and disturbed habitats. It belongs to the family Lamiaceae, and it is usually a weed that grows along wastelands, roadsides, rail tracks, etc.[6] The seeds or the nutlets are about 1.2 to 1.5mm long and are slightly notched at the end. They resemble the seeds of Ocimum species and get dispersed through wind, water, animals, or vehicles.[7] Due to their strong smell, the seeds are employed as an infertility agent in treating dysuria, as an insectifuge. It is also carminative, lactogenic, anti-catarrhal, anti-parasitic, and ulcer protective.[8,9] *Coccinia grandis* belongs to the family Cucurbitaceae and is also known as ivy gourd, little gourd, baby watermelon, gentleman's toe, and kundru.[10] It is native to Bengal and other parts of India and grown in Africa and Australia. The fruit is ovoid or elliptical, about 25-60mm long, 15-35 mm in diameter, and hairless. The seeds are tan colored and are 6-7mm long. Almost all the parts of this plant are found to be beneficial with a wide variety of actions.[11] The fruits are found to be hypoglycemic, analgesic, antipyretic, and hepato-protective in nature. Scientific evidence is to be produced for these activities. Although natural drugs are found to be safe, few drugs, when consumed for the long term, show toxicity. These plants are in wide use without adequate information on their toxicity profile. Therefore, the present study is intended to identify the major chemical constituents and evaluate the acute oral toxicity of the hydroalcoholic seed extract of *Hyptis suaveolens* and hydroalcoholic fruit extract of Coccinia *grandis.*

2. MATERIALS AND METHODS

2.1. Plant authentication and voucher number

The *Hyptis suaveolens* and the *Coccinia grandis* plants were collected from the local market, identified and authenticated by the botanist Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, S.V University, Tirupati, with voucher number 0709 and 0579 for *Hyptis suaveolens* and the *Coccinia grandis* respectively.

2.2. Plant Extraction

The freshly collected seeds of *Hyptis suaveolens (L.) Poit* and fruits of *Coccinia grandis* were shade dried and coarsely powdered. The powder was passed through sieve no.40, and the sieved powder was stored in an airtight container for further use. 100g of each dried plant material powder was initially macerated with 70%v/v hydroalcoholic for 7 days. It was then filtered, the solvent was evaporated, and the percentage yield was calculated and stored in desiccators until further use.[12,13]

Table I: Percentage yield and nature of plant extract

Plant Material	Solvent used	Texture	Percentage yield (%w/w)	Color	Nature
Hyptis suaveolens seeds	70%v/v hydroalcoholic	Sticky	6.5%	Dark brown	Semisolid
Coccinia grandis fruits	70%v/v hydroalcoholic	Sticky	4.7%	Dark brown	Semisolid

Table 1. shows the percentage of yield and nature of the extract after hydro alcoholic (60:40) extraction by maceration method of both *Hyptis suaveolens* seeds and *Coccinia grandis* fruits after as 6.5% and 4.7%, respectively, whereas *the* nature of both the extracts are semisolid, sticky and dark brown.

2.3. Phytochemical screening

The hydroalcoholic extracts of *Hyptis suaveolens* and *Coccinia grandis* are tested for the presence of different active phytochemicals, including alkaloids, carbohydrates, proteins, steroids, sterols, polyphenols, flavonoids, tannins, terpenoids, steroids, phenols, gums and mucilage, glycosides, saponins, terpenes, tannins and flavonoids using the method described by Jaiswal Bhagat Singh et al. [14]

2.3.1. Test for alkaloids

A small portion of the solvent-free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. With the filtrate obtained, the following tests were done:

a) **Mayer's test**: to 2ml of this filtrate, a few drops of Mayer's reagent were added along the sides of the tube. The formation of a white or yellow creamy precipitate indicates the presence of alkaloids.
b) **Hager's test:** To 2 ml of filtrate, a few drops of Hager's reagent were added to the test tube. The formation of yellow color indicates the presence of alkaloids.
c) **Wagner's test:** 2ml of this extract was taken in a test tube, and Wagner's reagent was added. A reddish-brown precipitate was observed, indicating the presence of alkaloids.

2.3.2. Test for Carbohydrates

a) **Fehling's test:** In a test tube, 1 ml of Fehling's solution A and 1 ml of Fehling's solution B were taken. To this mixture, 2 ml of test extract was added and mixed. It was then heated and boiled. The formation of red precipitate was observed, indicating the presence of carbohydrates.
b) **Benedict's test:** Equal volumes of Benedict's reagent and the extract were mixed in a test tube and heated for 5-10

minutes in a water bath. The solution changes to green, yellow, or red depending on the amount of reducing sugar in the test solution indicating the presence of reducing sugar.

2.3.3. Test for Proteins

a) **Biuret's Test:** When the extracts were treated with copper sulphate solution, followed by the addition of sodium hydroxide solution, the violet color indicates the presence of proteins.

2.3.4. Test for Triterpenes and Steroids

a) **Salkowski's test:** the extract was treated with chloroform and filtered. A few drops of concentrated sulphuric acid are added to the filtrate, shaken, and allowed to stand. If the lower layer turns red, it indicates the presence of steroids. The presence of a golden yellow layer at the bottom indicates the presence of triterpenes.
b) **Liebermann Burchard Test:** When the extracts were treated with concentrated sulphuric acid, a few drops of glacial acetic acid, followed by the addition of acetic anhydride, there is a formation of the violet ring in between the two layers, and the appearance of green color in the aqueous upper layer indicates the presence of steroids.

2.3.5. Test for Sterols

When the extracts were treated with 5%potassium hydroxide solution, the appearance of pink indicates the presence of sterols.

2.3.6. Test for phenol compounds

a) **Ferric chloride test:** When the extracts were treated with a neutral ferric chloride solution, the appearance of blue, green, or violet color indicates the presence of phenols.
b) **Lead acetate test:** Some amount of extract was dissolved in distilled water. To this solution, a few drops of lead acetate solution was added. The formation of a white precipitate indicates the presence of phenol compounds.

2.3.7. Test for Glycosides

a) **Borntrager's test:** To the 3 ml of extract, dilute sulphuric acid was added, boiled for 5 minutes, filtered, and cooled. An equal chloroform was added to the cold filtrate and shaken well. The organic solvent layer was separated, and ammonia was added to it. The formation of pink to red color in the ammonia layer indicates the presence of anthraquinone glycosides.
b) **Keller-killiani test:** When a pinch of the extracts was dissolved in the glacial acetic acid, and a few drops of ferric chloride solution was added, followed by the addition of concentrated Sulphuric acid, the formation of the red ring at the junction of two liquids indicated the presence of glycosides.

2.3.8. Test for Saponins and Tannins

a) **Foam Test:** 1 ml of extracts are diluted to 20 ml of distilled water and shaken well in a test tube. The formation of foam in the upper part of the test tube indicated the presence of saponins.
b) **Gelatin test:** 1% gelatin solution containing 10% sodium chloride was added to the test extract. The precipitate formation was observed, indicating the presence of tannins.

2.3.9. Test for Flavonoids

a) **Lead acetate test:** To extract few drops of lead acetate solution was added. The formation of a yellow precipitate may indicate the presence of flavonoids.
b) **Alkaline reagent test:** In a test tube, when a few drops of sodium hydroxide is added to the extract intense yellow color is formed, which becomes colorless in addition to a few drops of dilute acid, which indicates the presence of flavonoids.

2.4. Fixed oils & Fats

a) **Filter paper test:** small quantities of extracts were separately pressed between filter paper folds. The appearance of oil stains on the paper indicates the presence of fixed oils.

2.5. Toxicity studies

All the procedures for the acute oral toxicity studies were performed according to OECD guidelines for the testing of animals "Acute oral toxicity study" guideline no**.** 423 annex 2d[15] and The Institutional Ethical Committee approved the protocol for these experiments under number SVSMC/IAEC no.2/2020 /648/A (CPCSEA Registration in No.25/13/2014/CPCSEA, Dated 01-08-2018).

2.6. Experimental animals, housing, and feeding conditions

12 Healthy young adult female Wistar rats of 8 to 12 weeks old, nulliparous and nonpregnant and weighing around 150-180g were used for the study. Female Wistar rats are used because they are slightly more sensitive than males. They were maintained at the Experimental Animal House of the Department of Pharmacology at 22 ± 3° C with 30 - 70 % relative humidity. Animals were kept in a 12hours light & dark cycle with artificial lighting. Water and Pellet rodent feed were provided ad libitum in polypropylene rat cages (approximate internal dimensions of 370 mm × 210 mm × 150 mm) with Corn cob bedding (3 animals per cage). They were examined and acclimatized to the new environmental conditions before the start of the experiment 21 days. Before the start of the experiment, animals were thoroughly examined physically, and knew their state of health and suitability for the experiments.

2.7. Exclusion criteria

Pregnant adult Female Wistar rats weighing more than 180g and rats older than 12 weeks are excluded from the study.

2.8. Preparation of Animals

All 12 animals used in the study were randomly selected and marked individually for identification. Rats were kept for 5 days for acclimatization and were fasted overnight before dosing.

2.9. Dose preparation

According to OECD 423 guidelines, the volume of the test solution administered to rodents should not exceed more

than ml/100g of body weight. Keeping this in mind, the animals were weighed. The extracts of *Hyptis suaveolens* & *Coccinia grandis* at a single dose of 2000 mg/kg body weight were administered orally using an oral gavage tube to three animals in each group according to the body weight of the animals. The animals were kept fasting further for 3 to 4hrs. In acute toxicity testing, a step I is where we observe the animals for 48 hours immediately after dosing. The animals were observed for signs of toxicity, such as alterations in eyes, skin, fur, respiratory system, central nervous system, autonomic nervous system, behavioral pattern, etc. Also, the animals were observed for mortality. If there is 50% mortality, we will step down the dose to 300 mg/kg body weight, and if there is no mortality, we will proceed to step II, where we give the same dose to confirm step I and observe the animals up to14 days as per figure 1.[15]

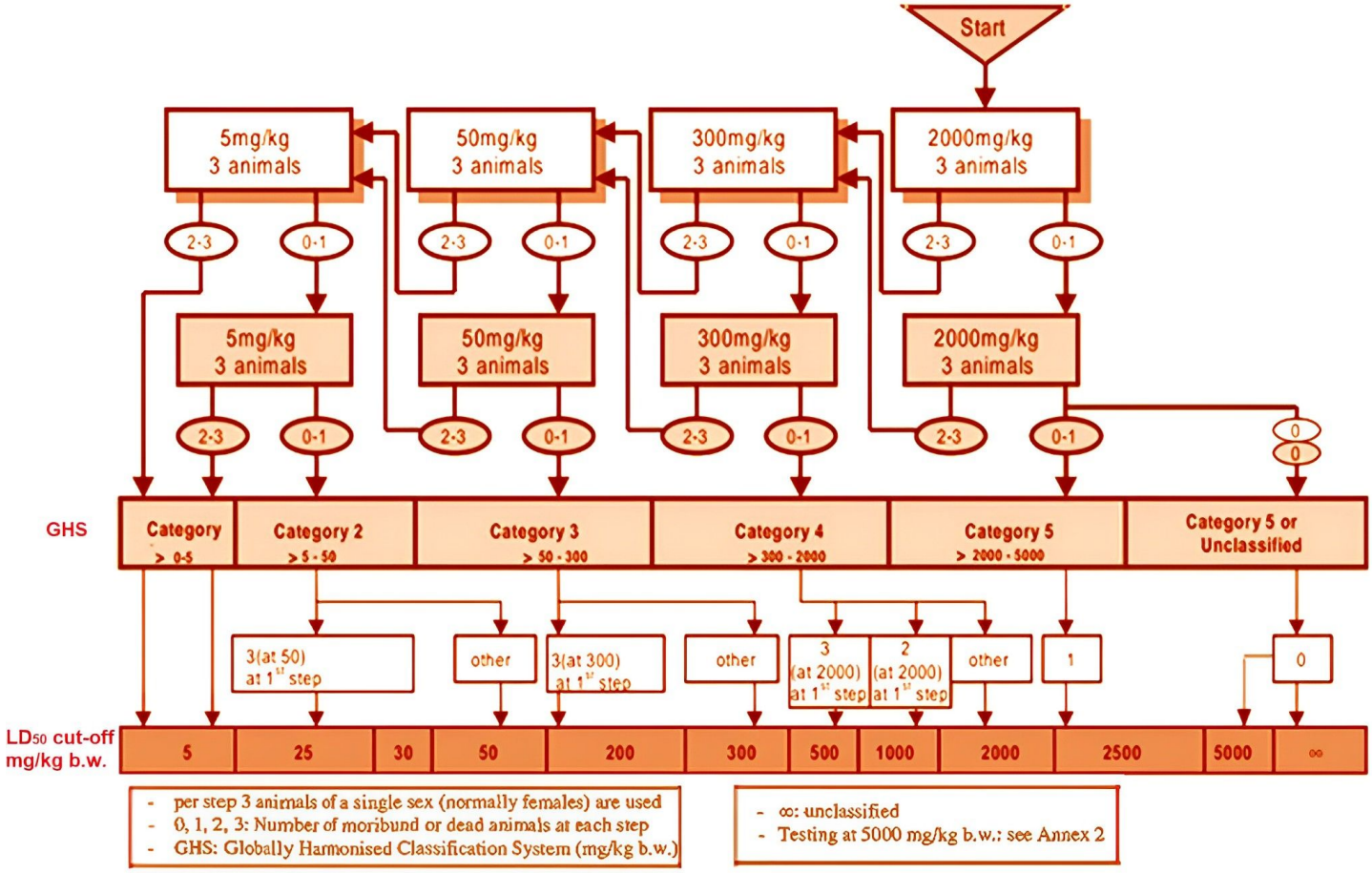

Fig I: Test procedure with a starting dose of 2000 mg/kg body weight.

2.10. Statistical Analysis

In the toxicity study, all the values are expressed as mean ± SEM using the software Graph pad prism version 6.

3. RESULTS

3.8. Preliminary phytochemical screening of the extracts

The hydroalcoholic extracts of seeds of *Hyptis suaveolens (L.) Poit* and fruits of *Coccinia grandis* were subjected to the phytochemical screening of both extracts for the presence of phytochemical constituents such as alkaloids, carbohydrates, proteins, steroids, sterols, polyphenols, flavonoids, tannins, terpenoids, steroids, phenols, gums and mucilage, glycosides, saponins, terpenes, tannins, and flavonoids are shown in table I. and Percentage yield and nature of plants extract is given in table II.

Table II: Phytochemical screening of hydroalcoholic extracts of seeds of *Hyptis suaveolens* and fruits of *Coccinia grandis*.

Test	Hydroalcoholic of *Hyptis suaveolens (L.) Poit seeds* Extract	Hydroalcoholic of *Coccinia grandis fruit* Extract
Alkaloids	+	+
Carbohydrates	+	+
Tannins	+	-
Flavonoids	+	+
Steroids & Terpenoids	-	+
Glycosides	+	+
Saponins	-	+

Phenols	+	+
Proteins	-	-
Fixed oils & Fats	+	-

+ = Present: - = Absent

Table I depicts the presence and absence of various phytochemical constituents in the hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruits.*

3.9. Acute Toxicity Study

All the animals were observed for the effect of hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruits* on the below parameters to evaluate the safety and significance of adverse effects for 14 days per the OECD procedure mentioned above in Figure I.

3.10. Body weight

The body weight of all the animals was measured on day 0 before the dosing, day 7, and day 14, and the results are shown in Table III.

Table III: Changes in the body weights of the animals throughout the study.

Drug group and *step*	Dose mg/kg b.w	Test day 0 (g)	Test day 7 (g)	Test day 14 (g)	The difference in b. w. in gram (day 0 to day 7) W7 – W0	The difference in b. w. in gram (day 0 to day 14) W14 – W0
HAHS - step-I	*2000*	168.66±2.30	170.47±2.36	166.92±1.90	1.81	-1.73
HAHS - step-II	*2000*	163.39±2.28	165.06±3.67	161.75±1.60	1.66	-1.60
HACG- step-I	*2000*	160.44±3.50	162.98±2.53	159.86±3.47	2.53	-0.58
HACG - step-II	*2000*	166.13±1.31	168.55±0.66	165.19±0.96	2.41	-0.94

Values are expressed in Mean±SEM: Number of animals per step =3 female rats; W7 = final weight on day 7 and W0 = initial weight on day 0; W14 = final weight on day 14; HAHS = Hydroalcoholic extracts of Hyptis suaveolens seeds; HACG = Hydroalcoholic extracts of Coccinia grandis fruit

Table III illustrates no abnormal change in body weight after administration of both extracts at a dose of 2000 mg/kg/b.w. The animals had gained body weight by day 7 compared to day 0, and the animals showed a slight loss in body weight by day 14 compared to day 0.

3.11. Clinical signs

All the animals were observed closely for their clinical signs with the following frequency per the critical toxicity assessments. Daily once during the acclimatization period, just before dosing, during the first 30 minutes after the dosing, and at approximately 1, 2, 3, and 4 hours after the dosing on day 0. Thereafter every day for 14 days. The following parameters were observed, condition of skin and fur, eyes and mucus membrane, respiratory, circulatory and autonomic, and central nervous system, somatomotor activity, and behavioral pattern. The results are shown in Table IV, V, and VI.

Table IV: Physical observations in the rat after administration of Hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruit* in a single dose of 2000 mg/kg b.w per step

Parameter observed	Hydroalcoholic extracts of Hyptis suaveolens seeds		Hydroalcoholic extracts of *Coccinia grandis fruit*	
	Step I	Step II	Step I	Step II
Skin color	Normal	Normal	Normal	Normal
Body weight	Normal	Normal	Normal	Normal
Fur color	Normal	Normal	Normal	Normal
Eye color	Normal	Normal	Normal	Normal
Respiration	Normal	Normal	Normal	Normal
Urine color	Normal	Normal	Normal	Normal
Temperature	Normal	Normal	Normal	Normal

Number of animals per step =3 female rats

Table IV shows that all the parameters physically examined after administering Hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruit* remained normal without any abnormal changes during the study period in both Steps I and Step II.

Table V: Sensory responses in the rat after administration of Hydroalcoholic extract of seeds of *Hyptis suaveolens* and fruits of *Coccinia grandis* in a single dose of 2000 mg/kg b.w per step)

Parameter observed	Animals treated with *Hyptissuaveolens*		Animals treated with *Coccinia grandis*	
	Step I	Step II	Step I	Step II
Touch response	Normal	Normal	Normal	Normal
Pain response	Normal	Normal	Normal	Normal
Sound response	Normal	Normal	Normal	Normal
Corneal reflex	Normal	Normal	Normal	Normal

Number of animals per step =3 female rats

Table V establishes No change in the touch responses, pain responses, rational responses, and corneal reflexes in both steps after administering plant extracts. In addition, all the animals remained normal and healthy throughout the experiment, and all the sensory responses remained unchanged.

3.12. General signs and symptoms

The general and behavioral parameters observed for acute toxicity signs after administering the plant extracts are shown in Table VI. No significant changes in behavior were observed in the treated groups following the acute toxicity study

Table VI: General signs and behavioral parameters in rats after administration of extracts.

Parameter observed	Animals treated with *Hyptis suaveolens*		Animals treated with *Coccinia grandis*	
	Step I	Step II	Step I	Step II
Alertness	Normal	Normal	Normal	Normal
Food intake	Normal	Normal	Normal	Normal
Sleep	Normal	Normal	Normal	Normal
Convulsions	Normal	Normal	Normal	Normal
Ataxia	Not present	Not present	Not present	Not present
Coma	Absent	Absent	Absent	Absent
Drowsiness	Absent	Absent	Absent	Absent
Breathing	Normal	Normal	Normal	Normal
Tremors	Absent	Absent	Absent	Absent
Irritability	Absent	Absent	Absent	Absent
Salivation	Normal	Normal	Normal	Normal
Lacrimation	Normal	Normal	Normal	Normal
Diarrhea	Normal	Normal	Normal	Normal

Number of animals per step =3 female rats: Dose = 2000 mg/kg b.w.per step

Table VI shows All rats treated with extracts recorded normal behavioral and General signs. The monitoring of general and behavioral parameters related to autonomic and central nervous system activities of treatment rats remained normal, and no abnormal changes were seen in both steps for 14 days of the experiment.

3.13. Mortality, moribund status, and macroscopic findings

Mortality and Moribund status were observed in the study as per OECD 423 guidelines. No mortality and Moribund status were observed in steps I and II for the entire study period. In addition, no macroscopic/gross pathological lesions were observed for all the animals after terminal sacrifice after a 14 days observation period. Both the results are shown in Table VII.

Table VII: Moribund status, mortality, and microscopic/gross pathological findings in the rat after administration of extracts

Step	Plant extract	Moribund status	Mortality	Microscopic/gross pathological findings after terminal sacrifice	
				External internal	External internal
Step I	*Hyptissuaveolens seeds*	Not present	Not present	NAD	NAD
	Hydroalcoholic of *Coccinia grandis fruit*	Not present	Not present	NAD	NAD
Step II	*Hyptissuaveolens seeds*	Not present	Not present	NAD	NAD
	Cocciniagrandis fruit	Not present	Not present	NAD	NAD

Number of animals per step =3 female rats: NAD= no abnormalities detected

Table VII reflects that all the animals are active and healthy with no sign of moribund status and mortality; the microscopic/gross pathological finding also showed no Abnormality after terminal sacrifice in Step I and Step II after administration of Hydroalcoholic extract of *Hyptis suaveolens* seeds and Coccinia grandis fruit.

4. DISCUSSION

In recent years herbal medicines are gaining a lot of importance as an alternative to conventional therapy[16] due to the availability of a wide range of lead compounds. As these herbal drugs are widely used by people without medical supervision, screening of plant products to evaluate the safety and identify the nature and significance of the adverse effect of any medicinal plant is considered an initial step[17,18] The present study was focused on the phytochemical screening and acute oral toxicity studies of the hydroalcoholic extracts from the seeds of *Hyptis suaveolens (L.) Poit* and fruits of *Coccinia grandis*. These extracts were prepared and stored in semi-solid form by freeze-drying method. The preliminary phytochemical screening of the seeds of *Hyptis suaveolens* reveals the presence of alkaloids, glycosides, flavonoids, carbohydrates, phenols, terpenes, tannins, and sterols(table: II). Kumkum Agarwal and Ranjana Varma[19] also observed the presence of alkaloids, carbohydrates, reducing sugars, flavonoids, glycoside, tannin, phenolic compounds, protein, and amino acids on phytochemical analysis of *Hyptis suaveolens* L. methanolic extract of whole plants Due to the presence of various pharmacological active constituents; the plant *Hyptis suaveolens* is said to have anti-inflammatory, analgesic, anti-oxidant,[20] antiplasmodial, anti-fertility, immunomodulatory, anti-ulcer [21]. The phytochemical screening of the fruits of *Coccinia grandis* also reveals the presence of different pharmacologically active constituents like alkaloids, glycosides, carbohydrates, terpenes, sterols, steroids, glycosides, phenols, flavonoids, and tannins (table: I). Literature has reported that the extracts of this plant, due to the presence of these phytochemical compounds, showed analgesic, anti-pyretic[22], anti-inflammatory, antimicrobial, anti-diabetic, anticancer[23], anti-malarial, anti-asthmatic activities[24,25]. The oral route administration is the most useful and commonly used route in a toxicity study. The animals need to fast before administering the plant extract because food and other chemicals in the digestive system may interfere with the reactions of the tested materials. All the procedures were performed based on the appropriate OECD guideline[26]. The 14-day oral toxicity study revealed that the hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruits* did not cause mortality or other behavioral and motor abnormalities in rats (Tables: VI&VII). Other physical or visual parameters were observed for 14 days. The temperature, skin color, and eye color were normal without any changes in all the animals (table: IV). The touch, pain, rational response, and corneal reflex were normal in all the animals (table: V). The animals had no diarrhea, coma, breathing difficulty, sedation, or tremors. Lacrimation and defecation were also normal. The animals were normal and showed normal signs (table: VI). Evaluating pathological abnormalities in organs is considered a basic test in the safety assessment of drugs or compounds to be tested[27]. In the present study, no abnormalities were detected in the gross pathological examination of the animal tissue. Overall, the animals were normal and showed normal signs. The body weight changes and feed consumption of rats were monitored for 14 days from the start of the acclimatization period as they are the important parameters that give the information of general health status of experimental animals[28]. The loss in body weight of more than 10% from the initial weight is considered a significant indicator of the adverse effects of drugs and chemicals.[29,30] In the present study, no animal showed signs of sudden weight loss or gained weight (Table III). All the rats' food and water intake followed a routine and general trend. It shows that the administration of extracts did not affect the animals' normal growth, metabolic growth, appetite, or general health. There was also no disturbance in carbohydrate, protein, or fat metabolism.[31] The results from this acute oral toxicity study suggested that the *Hyptis suaveolens seeds* and *Coccinia grandis fruits* extracts are relatively nontoxic, and the 2000 mg/Kg body is considered as no-observed-adverse-effect level (NOAEL) of extracts. However, the safety of prolonged use can be confirmed by conducting further toxicity studies, such as subacute, chronic, or genotoxic studies using repeated doses of *Hyptis suaveolens seeds* and *Coccinia grandis fruits*.

5. CONCLUSION

In the present study, the phytochemical screening and acute oral toxicity studies of hydroalcoholic extracts of *Hyptis suaveolens seeds* and *Coccinia grandis fruits* contain various pharmacologically active constituents indicating they can be evaluated for various pharmacological activities. Acute oral toxicity studies suggest that these extracts are relatively safe, and the acute oral LD50 is determined as 2000<ATE≤5000 mg/kg b.w. The LD50 cut-off value of the extracts falls under category '5' or unclassified with an LD50 cut-off value of 5000 mg/kg body weight according to the GHS classification system. Thus, the seeds of hyptis suveolens and fruits of coccinia grandis which are generally consumed, are presumed safe to be used as health supplements. However, subacute and chronic toxicity studies are to be done to understand the safety of repeated-dose administration further.

6. AUTHORS CONTRIBUTION STATEMENT

Jagannath Narumalla gathered the data and literature regarding the study, performed the experiments, and analyzed the data. Results and prepared the original manuscript of the paper. Dr. D. Sheela has conceptualized and designed the study. She also went through the original draft prepared by Jagannath Narumalla and gave valuable input in designing the manuscript. Dr. Rohit Dixit has helped gather related works data and analyzed the literature and results thoroughly. He has also gone through the prepared manuscript. All authors have read and approved the final version of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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