Analysis of phytochemical constituents, anthelmintic and insecticidal properties of leaf extracts of hyptis suaveolens (L.) Poit

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Abstract

Objective: The present study was aimed to evaluate the phytochemical, anthelmintic and insecticidal properties of leaf extracts Hyptis suaveolens.

Materials & Methods: Phytochemical constituents present in H. suaveolens were studied by analysis of different solvent extracts using standard methods. Anthelminthic assay was carried out for aqueous and methanol extracts against Pheretima posthuma. The insecticidal activity of aqueous extract was tested against Tribolium castaneum using filter paper impregnation method.

Results: Phytochemical screening results indicated the presence of carbohydrates, alkaloids, cardiac glycoside, caumarin glycoside, sapanoids, flavonoids, phytosterols, fat and oils, and phenols, tannins and terpenoids in different solvent extracts. The aqueous and methanol extracts showed anthelminthic activity against P. posthuma. The methanol extract showed the highest anthelmintic activity. The aqueous extract of H. suaveolens also showed insecticidal activity. On the third day, the insects were found to be paralyzed in the highest concentration (i.e. on 200 mg/ml) and after 96 hour onwards the extract showed good activity. After 144 hours, all the concentrations showed insecticidal activity.

Conclusions: H. suaveolens contains vast array of phytochemicals and the aqueous and methanol extracts showed anthelmintic activity. Aqueous leaf extract also showed insecticidal activity against the red flour beetle, T. castaneum.

Keywords: Activity, Antihelmintic, Extract, Hyptis, Insecticidal, Pheretima posthuma, Phytochemical, Tribolium castaneum.

Introduction

Hyptis suaveolens L.Poit. is a potent medicinal herb belongs to the family Lamiace ae. This plant is considered as one of the world's most noxious exotic invasive weed invading the natural ecosystems at an alarming rate. Besides these harmful effects of H. suaveolens on natural ecosystems as an invader weed, this plant is an important source of many pharmacological and industrial constituents.[1,2,3]

H. suaveolens has both medicinal as well as insecticidal properties and it is used for traditional medicine for the treatment of various illnesses.[4] The leaves of this plant reported to contain alkaloids, terpenes, phenols, tannins, flavanoids, and volatile oil.[5,4] Reported pharmacological activities of the plant include anti-inflammatory,[6] antiulcer,[7] antioxidant,[8] insecticidal,[9] antibacterial[10] and antifungal properties.[4] Leaves of plant have been traditionally used as a stimulant against cold and diarrhea.[11] Fumes of the dried leaves are also used to repel mosquitoes and control insect's pest of stored grains.

Helminth infections are a major health concern in developing and less developed countries because they predispose humans and cattle to many other infections such as bacterial and fungal infections.[12] Immature forms of the parasites invade human beings via the skin or gastrointestinal tract and evolve into well differentiated adult worms that have characteristics tissue distribution. Anthelmintics are drugs that may act locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminths or development forms that invade organs and tissues.[13] Anthelmintics from the natural sources may play a key role in the treatment of these parasite infections.[14] In ethnomedicine, at least 80% of the world's population in developing countries relies medicinal plants from time to time as powerful drugs in traditional or alternative healthcare systems.[15]

Aromatic plants are considered as the most efficient insecticides of botanical origin, and essential oils present in them often constitute the bioactive fraction.[16] In developing countries, Lamiaceae have traditionally been used for their insecticidal and repellent properties against several insects' species.[17] Studies revealed that H. suaveolens leaf extracts have insecticidal activity against different pests. Chemicals pesticides used for crop protection act as environmental pollutants and cause undesirable effects on animals and human beings. So the best alternate strategy is to develop and use ecofriendly insecticides of plant origin and recommend its widespread use. Among natural products, several botanical pesticides are effective and have favourable eco toxicological properties which make them potentially suitable for use in integrated pest management of different pest species.[18] The main objective was to further screen the presence of phytochemicals and to study the anthelmintic and anti-insecticidal properties of H. suaveolens.

Materials and Methods

Collection and identification of plant

H. suaveolens was collected from S.D.V. College of Arts and Applied Science campus, Alappuzha, Kerala, India. This plant material was identified by Dr. Shaji P.K., Scientist, Environmental Resources Research Centre (ERRC), P.B. No. 1230, P.O. Peroorkada, Thiruvananthapuram, Kerala state, India. Fresh leaves were collected and washed several times with water, shade dried and then pulverized to coarse powder in an electric grinder. The powder was then stored in air tight bottles for further studies.

Chemicals and drug used

All the solvents used for the extraction process were of analytical grade and procured from SD Fine Chemicals, Mumbai, India. Albendazole was procured from Cipla Limited, Mumbai, India. Concentrated sulphuric acid, ferric chloride, NaOH, ninhydrin, HCl and Tween 20 were purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India. All the other chemicals and reagents used were of analytical grade and were prepared in deionized water.

Analysis of phytochemical constituents

The phytochemical constituents present in H. suaveolens were extracted using different solvents (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) based on polarity and were tested using standard methods.[19,20,21,22]

Anthelmintic activity: preparation of plant extract

One hundred gram of dried leaf powder of H. suaveolens were weighed and extracted in sterile distilled water and ethanol separately. The powder was kept in sterile distilled water and also in ethanol for 24 hours in reagent bottles at room temperature and was filtered using Whatman filter paper No 1. The pH of the extract was adjusted to 7. These extracts were further diluted to 20, 40, 60, 80 mg/ml in normal saline containing Tween 20 (1%) and used for the experiment.

Worm collection and authentication

Pheretima posthuma (Indian earthworm) were purchased from The Little Flower Nursery and Organic Manures, Kalavoor, Alappuzha, Kerala, India and identified by Ms. Bindu P., Assistant Professor, and Department of Biotechnology, S.D.V. College of Arts and Applied Science, Alappuzha, Kerala. The worms were washed with normal saline to remove faecal matter before starting the experiment.

Preparation of standard drug

Albendazole was used as the standard drug and different concentrations such as 20, 40, 60, 80 mg/ml was prepared using normal saline diluted with Tween 20.

Anthelmintic assay

Five groups of nearly equal sized P. posthuma (consisting of two earth worms each in triplicates) was released in to 30 ml of experimental formulation kept in a petri dish. First group served as normal control which is treated only with normal saline, second group was treated with tween 20 along with normal saline served as negative control, third group serve as standard drug, containing Albendazole at varying concentrations of 20, 40, 60 and 80 mg/ml in Tween 20 (1%) diluted with normal saline. Extracts of methanol and water at different concentration (20, 40, 60 and 80 mg/ml) constituted the fourth and fifth group. All the test solutions and standard solutions were prepared freshly before starting the experiment. The mean time for paralysis was noted when no movement of any sort could be observed, except when the worm was shaken vigorously. The death time of the worms were recorded in minutes after confirming that worms neither moved when shaken nor when given external stimuli by putting motionless worms in 50 ^{o}C warm water.[23] Death was concluded when the worms lost their motility followed with white secretion and fading of their body colours.[24] The time for paralysis and death were expressed as mean ± Standard error for mean (± SEM). $P < 0.05$ - 0.01 were considered as statistically significant.

Insect Bioassay: collection and authentication of insect

Tribolium castaneum (red flour beetle) was collected from Narayana Store Kalarcode Junction, Alappuzha, Kerala, India and are used to evaluate the insecticidal property of H. suaveolens. The insects were identified by Ms. Bindu P., Assistant Professor in Zoology, Department of Biotechnology, S. D.V. College of Arts and Applied Science Alappuzha, Kerala, India.

Preparation of plant extract

Twenty grams of dried leaf powder of H. suaveolens was weighed and extracted in 100 ml sterile distilled water. The powder was kept in sterile distilled water for 24 hours in a reagent bottle at room temperature and was filtered using Whatman filter paper No. 1. The pH of the extract was adjusted to 7 and the aqueous extract was further diluted to 10, 50, 100, 150 and 200 mg/ml.

Insecticidal activity by filter paper impregnation method

Filter paper impregnation method described by EL-Kamali[25] was used to determine the mortality rate of the insects exposed to different concentrations of H. suaveolens. Filter paper was cut out in size of the petri plate and placed one filter paper in each of them. Two ml of aqueous extract was spread with the help of pipette over the filter paper. Sterile distilled water was taken as control. Ten insects were released into each petri plate. One ml of extract was added to the respective plates daily to prevent drying. Mortality rate were calculated after every 24, 48, 72, 96, 120, 144, 168,192 hours and expressed in percent mortality. The experiments were performed in the laboratory at 30ºC ± 0.5ºC. The percentage mortality observed was calculated using Abbott's formula[26]

(% test mortality - % Control mortality)/ 100 – Control mortality X 100

Results and Discussion

The qualitative phytochemical analysis showed the presence of carbohydrates, alkaloids, cardiac glycoside, caumarin glycoside, sapanoids, flavonoids, phytosterols, fat and oils, phenols, tannins and terpenoids in different solvent extracts. More phytochemicals were found in ethyl acetate and chloroform extract. Fats and oils and terpenoids were found in all extracts. Coumarin glycoside was present in all extract except chloroform. Protein was completely absent in all the different solvent extracts tested. The phytochemical constituents present in H. suaveolens are given in table 1.

Table 1: Phytochemical constituents of leaf extracts

Phytochemicals	Method	Solvent extracts ('+' indicate presence and '-' indicate absence)						
		Hexane	Chloro form	Dichloro-methane	Ethyl-acetate	Acetone	Methanol	Water
Carbohydrate	Molisch's test	-	+	+	-	-	-	-
Alkaloids	Wagners test	-	-	-	+	-	-	-
Cardiac Glycoside	Keller killiani test	+	+	-	+	-	+	-
Coumarin Glycoside	Alkaline test	-	-	+	+	+	+	+
Saponins	Foam test	-	+	-	-	-	+	+
Flavonoids	Alkaline reagent test	-	+	+	+	+	+	+
Phytosterols	Salkowski test	-	+	-	+	+	-	-
Fats & Oils	Spot test	+	+	+	+	+	+	+
Phenols	Ferric Chloride test	-	+	-	+	-	+	+
Tannins	Ferric Chloride test	-	+	-	+	-	+	+
Terpenoids	Borsche's reagent test	+	+	+	+	+	+	+
Proteins	Ninhydrin test	-	-	-	-	-	-	-

Percentage extractive

Different plant species would obviously have different chemical profile. Chemicals present in the plant material should be dissolved in different solvents for the purpose of further analysis. Ethyl acetate extract showed the highest percentage extractive value with 9.6 % and hexane extract (3.9 %) showed the lowest percentage extractive value. The percentage extractives of other extracts are given in table 2.

Table 2: Percentage extractives of different extracts

Solvents	Hexane	Chloroform	Dichloro methane	Ethyl acetate	Acetone	Methanol	Distilled Water
Percentage extractives (%)	3.9	4.1	5.5	9.6	6.2	7.6	4

Anthelminthic activity

The time taken for the paralysis and death vary with different concentrations in aqueous and methanol extracts. The aqueous extract exhibited poor anthelmintic property when compared to the standard drug, albendazole. The methanol extract exhibited the highest activity than albendazole and the time taken for the paralysis and death was very rapid. The methanol extract with 80% concentration took only 4 minutes for paralysis and 5 minutes for death. The lowest concentration of 20% exhibited paralysis of earth worms after 26 minutes and death after 34 minutes. The time taken for the death and paralysis of the methanol extract is lower than the standard drug (Table 3). The in vitro anthelmintic activity of ethanol and aqueous extracts of whole plant extracts of H. suaveolens against the Indian adult earthworm; P. posthuma and Ascardia galli were previously studied using piperazine citrate as positive and distilled water as negative control and reported significant anthelmintic activity at the highest concentration of 100mg/ml.[1,27]

Death was observed after 323 minutes in 20 mg/ml and 239 minutes in 80 mg/ml for the insects exposed to aqueous extract. Death was observed after 212 minutes in 20 mg/ml and 49 minutes in 80 mg/ml death was observed after when the earth worms are exposed to the standard drug, albendazole. The methanol extract showed the highest anthelmintic activity at 80 mg/ml and death was observed after 5 minutes. The corresponding values for different concentration of aqueous and methanol extracts are given in table 3. For anthelmintic bioassay the adult Indian earthworms P. posthuma was used because of its anatomical and physiological resemblance with the intestinal parasites of human beings.[28]

Table 3: Anthelmintic activity of the methanol and aqueous leaf extracts

Group	Extract	Concentration of extract/drug (mg/ml)	Response	
			Paralysis (in minutes)	Death (in minutes)
I	Normal Control	-	Nil	Nil
II	Negative Control	-	Nil	Nil
III	Standard (Albendazole)	20 40 60	163± 0.58 151± 1	212± 1 198± 0.58

		80	19± 0.58 16± 0	57± 0 48± 1.2
IV	Aqueous	20 40 60 80	248 ± 2.51 234± 1.52 203 ± 2.51 178± 2.51	323± 2.52 285± 0 263± 2 239± 9
V	Methanol	20 40 60 80	26 ± 0 7 ± 0 5± 0.58 4 ± 0	34± 1 10± 1 7± 0 5± 0.58

Insect bioassay

Aqueous extract of the plant were assessed for its toxic effects on T. castaneum which is an important pests of flour. For the first two days, there was no insecticidal activity by the aqueous extract against the insects in different concentrations (Table 4). On the third day, i.e. after 72 hours, two insects were found to be paralyzed in the highest concentration (i.e. on 200 mg/ml). After 96 hours, the next concentration has started to show their action on insects. As the hour passes, there occurs an increase in the number of paralyzed pest (Table 4). After 144th hour, all the concentrations tested showed insecticidal activity against the insects.

Table 4: Knockout stage of aqueous extract

Knockout / paralysis in time hours	Control (sterile distilled water)	Concentration of aqueous extract (mg/ml)				
		10	50	100	150	200
24	Nil	Nil	Nil	Nil	Nil	Nil
48	Nil	Nil	Nil	Nil	Nil	Nil
72	Nil	Nil	Nil	Nil	Nil	2
96	Nil	Nil	Nil	Nil	1	5
120	Nil	Nil	Nil	1	3	6
144	Nil	1	1	3	5	8
168	Nil	2	3	5	6	9
192	Nil	3	4	5	6	9

On the first and second day the percent mortality rate of T. castaneum was found to be nil. On the third day, some of the insets were found to be dead in the plate with the highest extract concentration (200 mg/ml) and the percent mortality rate obtained was 50%. The mortality increased as the day passed as shown in table 5. On the ninth day the percent mortality in different concentration, i.e. 10, 50, 100, 150 and 200 was found to be 10%, 25%, 35%, 45%, and 85% respectively.

Table 5: Percentage mortality of T. castaneum

Days	Control (sterile distilled water)	Concentration of aqueous extract (mg/ml)				
		10	50	100	150	200
1	Nil	Nil	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil	Nil	15
4	Nil	Nil	Nil	Nil	5	45
5	Nil	Nil	Nil	Nil	20	55
6	Nil	Nil	5	10	35	60
7	Nil	5	10	15	40	80
8	Nil	5	20	35	45	85
9	Nil	10	25	35	45	85

H. suaveolens has been reported to contain basic food nutrients: protein, carbohydrates, fats and fibre and phytonutrients such as alkaloids, tannins, saponins, flavonoids and terpenoids.[12] H. suaveolens also found to be effective against infestation by the pink stalk borer, Sesamia calamistis on maize and also for the control of Trogoderma granarium in stored groundnut .[9,29] The essential oil present in H. suaveolens has also been reported to be effective against the adult granary weevil Sitophilus granaries.[30] The ability of H. suaveolens to act as an effective insecticide or pesticide has been attributed to the presence of essential oils.

Conclusion

The preliminary qualitative phytochemical screening confirms the presence of phytochemicals with pharmacological significance. The methanol extract showed the highest anthelmintic activity when compared with standard drug albendazole. The aqueous extract also showed anthelmintic activity which was lower than that of methanol extract. The aqueous extract also showed insecticidal activity against T. castaneum which is a potent pest of flour.

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