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Analysis of Phytochemical Constituents, Anthelmintic and Insecticidal Properties of Leaf Extracts of *Andrographis paniculata*

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Abstract *Andrographis paniculata* is an annual herb distributed in tropical countries and it is widely used in traditional medicines. The present study was aimed to investigate the presence of phytochemical constituents, anthelmintic and insecticidal properties of leaf extracts of *A. paniculata*. The highest percentage of extractive of 24.6% was observed for ethyl acetate extract and lowest, 10.8% for hexane extract. The phytochemical constituents such as carbohydrates, alkaloids, coumarin glycoside, flavonoids, phytosterols, fats and oils and terpenoids were present in the different solvent extracts. The aqueous and methanol extract showed anthelmintic activity. Anthelmintic properties were tested in Indian earth worm, *Pheretima posthuma*. The methanol extract at 80 mg/ml concentration only took 3 minutes for paralysis and 5 minutes for death, the standard albendazole took 16 minutes for paralysis and 48 minutes for death. The aqueous extract took 73 minutes for paralysis and 79 minutes for death. Insecticidal activity of aqueous extract at 200 mg/ml concentration showed 40% mortality on the 4th day and 75% mortality was observed on 7th day. The aqueous and methanol extract showed the presence of anthelmintic activities and the aqueous extract showed considerable insecticidal property.

Keywords *Andrographis paniculata*, anthelmintic, extract, insecticidal, phytochemical

Introduction

Andrographis paniculata (Burm. f.) Wall. ex Nees is a traditional Chinese, Southeast Asian and Indian herb belongs to the family Acanthaceae is one of the highly used medicinal plants in the world. This plant is traditionally used for the treatment of common cold, diarrhoea, fever, jaundice, as a health tonic for the liver ailments, diabetes, hypertension, cardiovascular problems and cancer. *A. paniculata* is also reported to have antibacterial, antifungal and anthelmintic properties [1-4].

According to World Health Organization, about two billion people suffer from parasitic worm infections [5]. Helminthiasis is one of the most important human and animal diseases [6]. Most of the people in the third world countries are suffering from bacterial and helminthes infection, due to poor sanitation, poor unhygienic practices, malnutrition, and crowded living conditions [7] resulting in anaemia, malnutrition, eosinophilia and pneumonia. Control of helminths relies almost exclusively on a limited number of synthetic anthelmintic drugs. Therefore, novel and complementary helminth control options are urgently needed and phytochemical consttuents can play a major role in helminth control.

Traditional medicine uses plant extracts without taking into account the toxicity of the plants material. In the last two centuries, there have been serious investigations into the chemical and biological activities of plants. This has yielded compounds for the development of synthetic organic chemistry and the emergence of medicinal chemistry as a route for the discovery of more effective therapeutic agents [8]. The plant kingdom contains a huge array of chemical substances which are used by plants for their own defense against insect attack. These chemical substances may act as antifeedants, repellents, growth inhibitors, attractants, and chemosterilants or as insecticides [9-11]. The red flour beetle, *T. castaneum* is a key pest of stored food materials. It is estimated that 10 to 25% of global food production loss occurs from insect, microbial weakening and other factors annually [12]. This species has been found associated with a wide range of commodities including grain, flour, peas, beans, cacao, nuts, dried fruits and spices and milled flour [13].

The adult as well as the larvae of *T. castaneum* damage sound grains effecting quality and quantity of grains [14]. In case of serious infestation the grains and flour turns yellowish, stuffy and has a pungent, disagreeable smell which becomes unfit for human consumption. Control of these insects relies heavily on the use of synthetic insecticides and fumigants, which has led to problems such as disturbances of the environment, increasing costs of application, pest resurgence, resistance to pesticides and lethal effects on non-target organism in addition to direct toxicity to users [15,16]. Efforts have been made by researchers on the use of plant products as a prospective source of bioactive chemical compounds as commercial pest control agents and as a viable substitute for synthetic pesticides [17]. The purpose of this investigation was to evaluate the phytochemical constituents present in *A. paniculata* leaf, to screen the aqueous and methanol extracts for its antihelmithic potential and insecticidal properties of aqueous extract against red flour beetle *T. castameum.*

Materials and Methods

Collection and Identification of Plant

The plant, *Andrographis paniculata* (Burm.f.) Wall. ex Nees 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. The plant leaves were 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

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. Concentrated sulphuric acid, NaOH, ninhydrin, ferric chloride, 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

Analysis of the plant for various phytochemical constituent present were carried out for different solvent (hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol and water) extracts using standard methods [18-21].

Test for Carbohydrates

Molisch's test was performed to detect carbohydrates. Few drops of alcoholic solution of alpha naphthol were added to the extracts. Then, added 1 ml of concentrated sulphuric acid along the sides of the test tube. Formation of violet ring at the junction of the liquids indicated the presence of carbohydrates.

Test for Alkaloids

Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicated the presence of alkaloids.

Test for Cardiac Glycoside

Keller-Kelliani test was performed to detect cardiac glycoside. Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated H_2SO_4. A

brown ring of the interface indicated a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Test for Coumarin Glycoside

Ten percent NaOH was added to the extract and few drops of chloroform was also added. Observation of yellow colour indicated the presence of coumarin.

Test for Saponins

Foam test was performed to test the presence of saponins. To 2 ml of extract, added 6 ml of water in a test tube and was shaken vigorously. Formation of persistent foam confirmed the presence of saponins.

Test for Flavonoids

Alkaline reagent test was performed to test the presence of flavonoids. Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for Phytosterols

Salkowski test was used to detect phytosterols. To 2 ml of aqueous extract, 2ml of chloroform and 2 ml of concentrated H_2SO4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for Fats and Oil

Spot test was performed for fats and oils. The test solution was spotted on a filter paper with the test solution and the presence of oil staining on the filter paper indicated the presence of fixed oil and fats.

Test for Phenols and Tannins

Crude extract was mixed with 2 ml of 2% solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for Proteins

Ninhydrin test was employed to detect the presence of proteins. Crude extract when boiled with 2 ml of 0.2 % solution of ninhydrin, violet color appeared suggesting the presence of amino acids and proteins.

Test for Terpenoids

One ml of the extract was treated with Borsche's reagent (2,4-dinitrophenyl hydrazine in methyl alcohol) and 1ml of 3M HCl. Formation of orange colour indicated the presence of terpenoids.

Anthelmintic Activity

Preparation of Plant Extract

One hundred grams of shade dried leaf powder of *A. paniculata* were weighed and kept in sterile distilled water and also in methanol 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 and 80 mg/ml in normal saline containing tween 20 (1%) and used for further studies.

Collection and Authentication of Worms

Indian earth worm, *Pheretima posthuma* was procured from The Little Flower Nursery and Organic Manures, Kalavoor, Alappuzha, Kerala State and were further 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 and water to remove all fecal matter.

Preparation of Standard Drug

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

Anthelmintic Assay

Five groups of nearly equal sized *P. posthuma* (consisting of two earth worms each in triplicates) was released into 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 served as standard drug, containing albendazole at varying concentrations of 20, 40, 60, 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 the 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 given external stimuli by putting motionless worms in 50 ^{o}C warm water [22]. Death was concluded when the worms lost their motility followed with white secretion and fading of their body colours [23]. Values 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

T. castaneum (red flour beetle) was collected from Narayana Store Kalarcode Junction, Alappuzha, Kerala, India and are used to evaluate the insecticidal property of *A. paniculata*. 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 *A. paniculata* 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 [24] was used to determine the mortality rate of the insects exposed to different concentrations of *A. paniculata*. 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 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 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 [25]

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

Results and Discussion

A. paniculata is an important medicinal plant in the Indian Pharmacopoeia and it is widely used in ayurvedic formulations. The leaves and roots are traditionally been used over the centuries in Asia and Europe as a folklore medicine for a wide variety of ailments or as herbal supplements for various medicinal preparations.

Phytochemical Constituents

The qualitative phytochemical analysis for carbohydrates, alkaloids, cardiac glycoside, caumarin glycoside, sapanoids, flavonoids, phytosterols, fat and oils, phenols and tannins, proteins, and terpenoids were performed for hexane, chloroform, ethyl acetate, dichloromethane, acetone, methanol and aqueous leaf extract of *A. paniculata*. The results indicated the presence of carbohydrates, alkaloids, flavonoids, phytosterols, fats and oils and terpenoids. This result is in agreement with the observations made by Rajalakshmi and Cathrine [26] and Adekunle and Ayodele [27]. More number of phytochemicals was found in ethyl acetate extract such as carbohydrates, alkaloids, flavonoids, phytosterols, fat and oils in different solvent extracts. Phytochemical constituents such as cardiac glycoside, sapanoids, proteins, phenols and tannins were completely absent in all the solvent extracts. The findings of phytochemical constituents are given in table 1.

Table 1: Phytochemical constituents present in *A. paniculata*

Phytochemicals	**Solvent extracts ('+' indicate presence and '-' indicate absence)**							
	Methods	**Hexane**	**Chloroform**	**Dichloro methane**	**Ethyl acetate**	**Acetone**	**Methanol**	**Water**
Carbohydrate	Molish's test	-	-	-	+	+	-	-
Alkaloids	Wagner's test	+	-	-	+	-	+	-
Cardiac Glycoside	Keller killani test	-	-	-	-	-	-	-
Coumarin Glycoside	Alkaline test	-	-	-	-	-	-	+
Saponoids	Foam test	-	-	-	-	-	-	-
Flavonoids	Alkaline Reagent	-	-	-	+	+	+	+
Phytosterols	Salkowki test	-	+	+	+	+	-	-
Fats & Oils	Spot test	+	+	+	+	+	-	+
Phenols	$FeCl_3$ test	-	-	-	-	-	-	-
Tannins	$FeCl_3$ test	-	-	-	-	-	-	-
Proteins	Ninhydrin test	-	-	-	-	-	-	-
Terpenoids	Borsche's test	+	+	+	-	-	-	+

Percentage extractive

Different plant species would have different chemical profile. Chemicals present in the plant material could be dissolved in different solvents for the purpose of further analysis. Therefore, seven solvents such as hexane, chloroform, dichloromethane, ethyl acetate, acetone, methanol, and distilled water were selected to determine the soluble substance. Percentage extractives of different solvent extracts are given in table 2. Ethyl acetate extract showed the highest percentage extractive and acetone extract showed the lowest percentage extractive.

Table: 2. Percentage extractive of *A. paniculata*

Solvents	Hexane	Chloroform	Dichloro methane	Ethyl acetate	Acetone	Methanol	Distilled Water
Percentage extractive	10.8	12.4	14.3	24.6	10.3	11.6	11.3

Anthelmintic property

The aqueous and methanol extracts showed considerable anthelmintic activities when compared to standard drug, albendazole. The time taken for the paralysis and death varied with different concentrations. The aqueous extracts of 80% concentration showed activity within 73 minutes for paralysis and 79 minutes for death. The control drug, albendazole showed 16 minutes for paralysis and 48 minutes for death. The anthelmintic activity of aqueous extract increased with increase in concentration and it showed poor anthelmintic property when compared to the standard drugs. The results for anthelmintic activity of other concentrations are given in table 3. Zaridah et al. [28] reported that the aqueous extract of dried *A. paniculata* leaf was found to be active against adult worms of *Brugia malayi in vitro*.

Table: **3.** Anthelmintic activity of leaf extract of *A. paniculata*

Group	**Extract**	**Dose (mg/ml)**	**Response**	
			Paralysis (min)	**Death (min)**
I	Normal Control	-	-	-
II	Negative Control	-	-	-
III	Standard (Albendazole)	20	163± 0.58	212± 1
		40	151± 1	198± 0.58
		60	19± 0.58	57± 0

		80	16± 0	48± 1.2
IV	Water	20	184± 1	218± 3.6
		40	172± 2.08	183± 0.58
		60	118± 1.53	124± 3.6
		80	73± 3	79± 3.51
V	Methanol	20	62± 1.5	65± 2
		40	24± 0.2	29± 0.15
		60	8± 0.12	11± 1
		80	3± 0	5± 0.5

The methanol extract exhibited the highest activity than the standard drugs. The methanol extract of 80% concentration took only 3 minutes for paralysis and 5 minutes for death. Whereas the standard drug albendazole showed 16 minutes for paralysis and 18 minutes for death. The time taken for the death and paralysis of the methanol extract is lower than that of standard drug for all the other concentrations. The results for other concentrations are given in table 3. Padma et al. [29] evaluated the aqueous and methanol extracts for *in vitro* anthelmintic activity against adult earth worms *P. posthuma* and reported significant anthelmintic activities in concentrations of 25 mg/ml, 50 mg/ml, and 75 mg/ml.

The substance which destroys or prevents the development of parasitic worms, such as filariae, flukes, hookworms, pinworms, roundworms, schistosomes, tapeworms, trichinae, and whipworms are termed as anthelmintic drugs. An anthelminthic drug may interfere with the carbohydrate metabolism, inhibit their respiratory enzymes, block the neuromuscular action, or make them susceptible to destruction by the host's macrophages in parasites. Andrographolide (a colourless or light yellow crystal compound with a very bitter taste) is reported to be the main active constituent in *A. paniculata* and the presence of andrographolide is also attributed with some other activities like liver protection anticancer activity anti-diabetic activity and anti-malarial activity [30,31].

Insecticidal Activity

The aqueous extract of leaf of *A. paniculata* showed insecticidal activity against *T. castenum*. For the first three days there was no action by the extract against the insects. On the fourth day i.e. 96th hour 4 insects were found to be paralysed in the highest concentration (i.e. 200 mg/ml). After fifth day all concentrations except 10 mg/ml have started to show their activity. As the hour passes, there occurs an increase in the number of paralysed pest. After sixth day all the concentrations showed action against the insects (table 4).

Table 4: Toxicity of aqueous extract against *T. castenum*

Knockout / paralysis	**Concentration (mg/ml)**				
of insects days	**10**	**50**	**100**	**150**	**200**
1	Nil	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil	Nil
4	Nil	Nil	Nil	Nil	4
5	Nil	1	1	4	5
6	1	2	3	4	7
7	1	1	4	5	8

During the first three days, the percent mortality rate of *T. castenum* was found to be nil and on the fourth day onwards, some of the insets were found to be dead in the plate having highest concentration (200 mg/ml). The percent mortality rate obtained was 40%. The mortality rate increased as the day passes as shown in the table 5. Plant extract in all concentrations exhibited their lethal effect from sixth day onwards. On seventh day, the percent mortality at different concentration i.e., 10 , 50 ,100, 150 , 200 was found to be 5%, 10%, 40%, 45%, 75% respectively. The insecticidal activities of all the other extracts are given in table 5.

Table: 5. Percentage mortality of *T. castenum*

Death of insects	Concentration (mg/ml)				
in days	10	50	100	150	200
1	Nil	Nil	Nil	Nil	Nil
2	Nil	Nil	Nil	Nil	Nil
3	Nil	Nil	Nil	Nil	Nil
4	Nil	Nil	Nil	Nil	40
5	Nil	5	10	35	50
6	5	15	20	35	70
7	5	10	40	45	75

In addition to insecticidal activity, the crude methanol fractions of aerial parts of *A. paniculata* were reported to have growth inhibitory and oviposition deterrent activity against larval and adult stages of Bihar hairy caterpillar, *Spilarctia oblique* (Arctiidae). The methanol extract had the highest growth inhibitory activity and the diterpene andrographolide, displayed significant growth inhibitory, antifeedant properties and the ethyl acetate fraction possessed the highest oviposition-deterrent activity [32]. The diterpene andrographolide may also be responsible for the insecticidal activity.

The incorporation of natural plant products and their analogues into the management of agricultural stored insect pest has been considered as alternatives to synthetic products. This is mainly due to the fact that they are less detrimental to environment, economical and cheaper than synthetic chemical insecticides. Synthetic insecticides are noxious to man and livestock and can be pollutants to the environment. They may not be readily available and are un-affordable by the rural farmers. They may also be persistent in the produce [33]. So the best alternative is to encourage the extensive use of plant based botanicals to contain infestation of pests in stored grains.

Conclusion

The phytoconstituents such as carbohydrates, alkaloids, flavonoids, phytosterols, fats and oils and terpenoids were present in the different solvent extracts tested. The aqueous and methanol extract showed the presence of anthelmintic activities and aqueous extract showed considerable insecticidal property. It is concluded that the extracts of *A. paniculata* has the potential to control helminths and pests of stored grains.

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