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Evaluation of Anthelmintic Activity of isolated Piperine and Hydroalcoholic Extract from Pepper

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Abstract:
The present study deals with Phytochemical and Anthelminthic evaluation of fruits of Piper nigrum. Extraction of Piper nigrum was investigated for their Anthelmintic activity against PheritimaPhostima at various concentrations(200-400mg/30ml) of each extract was tested in paralysis time and the death time of the organisms. It was performed invitro screening against PheritimaPhostima.Theethanolic extract exhibited a maximum anthelminthic activity comparable to standard drug Albendazole (20mg/ml) and isolated Piperine. The hydro alcoholic (3:2)andhydro alcoholic extracts(1:1) shows modest activity. The preliminary phytochemical analysis indicated the presence of various phytoconstituents in all tested extracts. In proceeding to the research approach literature revealed the toxic nature of other chemical agent, so work directed towards herbal and phytomedicine.Alkaloids shows significant cidal effect on pheritimaphostima. ThePiperine alkaloid was isolated authenticated and characterized by TLC andSpectroscopic methods respectively.
Keywords:
Phytochemical, Piper nigrum, anti-helminthic activity.

1. INTRODUCTION

1.1 Helminthiasis

Helminth infections are among the most widespread infections in humans, distressing a huge population of the World. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health. It is an infestation with one or more intestinal parasitic worms' roundworms (Ascaris lumbricoides), whipworms (Trichuris trichuria) or hookworms (Necator americanus and Ancylostoma duodenale). Infected people excrete helminth eggs in their faeces, which then contaminate the soil in areas with inadequate sanitation. Other people can then be infected by ingesting eggs or larvae in contaminated food, or through penetration of the skin by infective larvae in the soil (hookworms). Infestation can cause morbidity and sometimes death by compromising nutritional status, affecting cognitive processes, inducing tissue reactions, such as granuloma, and provoking intestinal obstruction or rectal prolapse. The type and severity of symptoms is determined by the type of worm and the part of the body infected. The symptoms for Helminthiasis include - abdominal pain, diarrhoea, fever, fatigue, enlarged liver, enlarged spleen, cough, eosinophilia, asymptomatic gastrointestinal inflammation, mal absorption, bowel obstruction, anaemia, dehydration, bloody diarrhoea, chest pain, vomiting, constipation, weight loss, distended abdomen, itchy skin, eye symptoms, malaise, headache, itchy anus. The primary cause of Helminthiasis is - transmission of an infectious disease. Some subtypes of this disease are contagious - spread easily between people, while other subtypes are infectious – transmitted by a pathogenic organism[1].

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1.2 Anthelminthics

An anthelmintic is a substance that expels or destroys gastro-intestinal worms. The more common name is "dewormer" or "wormer". Anthelmintics are also called parasiticides, endectocides, nematodicides, parasitics, antiparasitics, and drenches. This includes both flat worms, e.g., flukes and tapeworms and round

worms, i.e., nematodes[2]. All anthelmintics essentially kill worms by either starving them to death or paralyzing them. Because worms have no means of storing energy, they must eat almost continuously to meet their metabolic needs. Any disruption in this process results in energy depletion. Interfering with feeding for 24 hours or less is sufficient to kill most adult parasites. Parasites will also die if they become paralyzed and temporarily lose their ability to maintain their position in the gut. The World Health Organization estimates that a staggering 2 billion people harbour parasitic worm infections parasitic worms also infect livestock and crops, affecting food production with a resultant economic impact. Also of importance is the infection of domestic pets. Indeed, the companion animal market is a major economic consideration for animal health companies undertaking drug discovery programmes[3]. Before 1940, the only compounds used to deal with parasitism were natural substances that had some effect on parasites, but also risked toxicity to the animal. The modern age of deworming began with the introduction of phenothiazine, which was administered to sheep as a drench and/or included in salt mixtures. It was sometimes combined with lead arsenate to control tapeworms. In the 1960s and 70s, organophosphate anthelmintics were introduced. Haloxon (Loxon) was an organophosphate anthelmintic that was eventually removed from the U.S. market due to toxicity issues, especially with Suffolk sheep and Angora goats. Nowadays, anthelmintics are separated into classes on the basis of similar chemical structure and mode of action. Although anthelmintics are sold under many brand names, there are only three chemical classes of dewormers.

A. Benzimidazoles

They bind to a specific building block called beta tubulin and prevent its incorporation into certain cellular structures called microbutbules, which are essential for energy metabolism. Interfering with energy metabolism is a much more basic mode of activity than the other classes of dewormers. For this reason, benzimidazoles are also able to kill worm eggs. Benzimidazoles have a wide margin of safety and broad spectrum activity.

B. Nicotinic Agonists

Nicotinic agonists comprise the next class of anthelmintics. They include imidazothiaoles (IMID) and tetrahydropyrimidines (TETR). The tetrahydropyrimidines group includes pyrantel pamoate (Strongid®), pyrantel tartrate, and morantel tartrate (Rumatel®). The tetrahydropyrimidines mimic the activity of acetylcholine, a naturally occuring neurotransmitter that initiates muscular contraction. The worm is unable to feed and quickly starves. Tetrahydroyrimidines only affect adult populations of worms. They do not have activity against the larval stages and are ineffective against cestodes (tapeworms) and trematodes (liver flukes). Imidazothiaoles have a similar mode of action as pyrantel and morantel, causing spastic paralyis of the worms. The group includes the drug levamisole (Prohibit®, Tramisol®, and Levasol®). In addition to being used as an anthelmintic for animals, levamisole has been used to treat various human diseases: colon cancer, melanoma and head and neck cancer, and influenza. It was discovered in 1966.

C. Macrolytic Lactones

Macrolytic lactones consist of two closely related chemical groups: avermectins and milbemycins. The avermectins include ivermectin (Ivomec®) and derivatives: doramectin (Dectomax®) and eprinomectin (Eprinex®) . Moxidectin (Cydectin®, Quest®) is the only milbemycin. All of the macrolytic lactone compounds have the same mode of action. They are developed from the same genus of soil dwelling-organisms (genus Streptomyces). They interfere with GABA-mediated neurotransmission, causing paralysis and death of the parasite. Macrolytic lactones are the most potent killer of worms and are more persistent in their effect. The duration of persistant activity varies according to the drug and formulation. Macrolytic lactones also have the unique quality of killing several external parasites such as lice, mites, and ticks. They have a wide margin of safety for livestock and are effective against all stages of worms, including inactive forms. However, macrolytic lactones are ineffective against cestodes (tapeworms) and trematodes (liver flukes). Ivermectin is also used as a broad-spectrum antiparasitic agent in humans.

1.2.1 Anthelminthic Resistance

Anthelmintic resistance was inevitable. It is a worldwide problem, having reached catastrophic proportions in some regions. Each time an anthelmintic is administered to an animal, it eliminates parasites whose genotype renders them susceptible and selects for parasites who are resistant and pass their resistant genes onto the next generation of worms. Certain practices accelerate the rate by which the worms become resistant to the anthelmintic(s). These include frequent deworming, treating every animal in the flock, putting treated animals immediately onto a clean pasture, underdosing the drug, injecting the drug and pouring the drug on the animal's back. Frequent treatments are primary cause of resistance. Understanding how anthelmintics work may help to devise strategies

for slowing down the rate by which the worms develop resisistance. At the same time, producers need to limit their use of anthelmintics in order to prolong their effectiveness for as long as possible. The level of drug resistance can be determined by performing the faecal egg count reduction test (FECRT) or by a larval development assay (LDA, DrenchRite®). In fact, anthelmintics can only be properly used if their effectiveness (or lack of effectiveness) is known[4].

1.3 PIPER NIGRUM

Piper nigrum, known as marica, is a plant in the family piperaceae. The fruit, known as a peppercorn when dried, is approximately 5 millimetres (0.20 in) in diameter, dark red when fully mature. Black peppers are native to India and are extensively cultivated there and elsewhere in tropical regions. Currently Vietnam is by far the world's largest producer and exporter of pepper, producing 34% of the world's Piper nigrum crop.

Kingdom	:	Plantae
Family	:	piperaceae
Genus	:	Piper
Species	:	nigrum
Binomial name	:	Piper nigrum

Chemical Composition of Black Pepper

- a-thujone(0.22-3.59%)-(terpenes)
- a-pinene(1.11-16.20%)
- Piperine(5-9%)
- camphene(0.23-1.44%)
- sabinene(0.14-13.78%)
- b-pinene(4.92-14-33%)
- a-phellandrene(0.46-27.37%)
- myrcene(1.66-2.53%)
- limonene(16.41-24.36%)
- caryophyllene(9.39.-30.94%)-(sesquiterpenes)
- B-farnesene(0.03-3.26%)
- B-bisabolene(0.09-5.18%)
- linalool(0.04-0.25%)
- terpinen-4-ol (0.01- 0.18%)

Uses of Piperine

- Anticonvulsant, anti-epileptic action.
- Increasing the adrenal glands' production of epinephrine (adrenaline)
- Altering contractions in the upper and lower digestive tract.
- Reducing the stomach's production of acid (for about 1 hour).
- Decreasing ulceration of the stomach.
- In Mexico it is used to treat stomach pain and malaria.
- Increasing the pancreas's production of digestive enzymes (amylase, lipase, trypsin and chymotrypsin)
- In Morocco used to treat weight loss and leukemia.
- Stimulating production of melanin
- Reducing inflammation due to irritation or allergy .
- Stimulating perspiration, which in effect causes a cooling of the body.
- Piperine can be found in most insecticides, particularly those that killed the common housefly.
- Used in the treatment of skin colour disorders and skin cancer .
- Piperine increases the bioavailability of many substances .
- Treatment for Snake Venom Poisoning.

2. MATERIALS

2.1 DRUGS AND CHEMICALS

Albendazole (Micro Labs.Ltd., Goa),Ethanol,10%AlcoholicKOH,1%Acacia and Normal Saline.

2.2 ANIMALS

Healthy adult Indian earthworm, Pheritima postuma was used for evaluating the anthelmintic activity due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings12. All earthworms were of approximately equal size. They were collected from local place and kept in a container.

3 METHODOLOGY

3.1 ISOLATION OF PIPERINE FROM BLACK PEPPER BY USING ETHANOL UNDER REFLUX

Placed 200g f ground black pepper in a 1000 ml round bottomed flask,add 600 ml of 95% ethanol and 5 boiling chips, and heat at reflux for 2h.Filtered the mixture by suction filteration and then concentrate the filterate by simple distillation.100ml of 10% alcoholic potassium hydroxide was added to the concentrate and the solution was stirred continuously for 30 min.The obtained solution was heated and water was added drop wise until yellow precipitate was formed. water was added until no more precipitate appeared to form and this was allowed to settle overnight. Needles of piperine were observed to be separated out. The solid was collected and recrstallized by using acetone. The product was packed in air tight container and used for further studies on phytochemical screening and pharmacological activity.

3.2 ISOLATION OF PIPERINE FROM BLACK PEPPER BY USING ETHANOL AND WATER (3:2) BY MACERATION

Placed 200g of ground black pepper in a 1000 ml round bottomed flask,add 360 ml of 95% ethanol,240 ml of water and kept for cold maceration .Filtered the mixture by suction filteration and then concentrate the filterate by simple distillation.100ml of 10% alcoholic potassium hydroxide was added to the concentrate and the solution was stirred continuously for 30 min.The obtained solution was heated and water was added drop wise until yellow precipitate was formed.water was added until no more precipitate appeared to form and this was allowed to settle overnight. Needles of piperine were observed to be separated out. The solid was collected and recrstallized by using acetone. The product was packed in air tight container and used for further studies on phytochemical screening and pharmacological activity.

3.3 Isolation of Piperine from Black Pepper by using Ethanol and Water (1:1) by Macceration:

Placed 200g of ground black pepper in a 1000 ml round bottomed flask,add 360 ml of 95% ethanol, 240 ml of water and kept for cold maceration. Filtered the mixture by suction filteration and then concentrate the filterate by simple distillation. 100ml of 10% alcoholic potassium hydroxide was added to the concentrate and the solution was stirred continuously for 30 min. The obtained solution was heated and water was added drop wise until yellow precipitate was formed. Water was added until no more precipitate appeared to form and this was allowed to settle overnight. Needles of piperine were observed to be separated out. The solid was collected and recrstallized by using acetone. The product was packed in air tight container and used for further studies on phytochemical screening and pharmacological activity.

3.4 PHYTOCHEMICAL SCREENING

Detection of glycosides: Extracts were hydrolysed 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. No Formation of rose pink colour in the ammonical layer indicates the absence of anthranol glycosides.

ii. Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. No Formation of pink to blood red colour indicates the absence of cardiac glycosides.

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 was not observed which indicates the absence of saponins. ii. Foam Test: 0.5 gm of extract was shaken with 2 ml of water. Foam was not observed which indicates the absence of saponins.

Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

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

ii. Wagner's Test: Filtrates were treated with Wagners reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

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

iv. Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution).
presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of tannins

i. Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. White precipitate was not formed confirming the absence of tannins.

3.5 Anthelmintic Activity

The anthelmintic activity was evaluated on adult Indian earthworms by the reported methods with slight modification[22]. 50ml formulations containing piperine in five different concentrations of aqueous and hydroalcoholic extracts of its various fractions (100, 150, 200, 300, 500 mg/ml) were prepared and six worms (same type) were placed in it. The wide range of dose was taken to establish the relationship between dose and pharmacological activity and also to find out the minimum and maximum dose that can be better therapeutically effective in compar-

ison to standard drug. Observations were made for the time taken to paralyse and/or death of individual worms. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.

4. Results

Phytochemical investigation of ethanol and hydroalcoholic extracts of fruits of black pepper prepared by under reflux and maceration showed the presence of alkaloid (piperine) (Table 2).

Anthelmintic activity of piperine was confirmed by examining the time taken for paralysis(P) and death (D) for Pheretima posthuma worms (Table 2). The assay was performed on adult Indian earthworm, Pheretima posthuma due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings.

Table 1: Phytochemicals detected

Phytochemicals	Under reflux	Maceration	
	Ethanol (2hours)	350 ml ethanol + 240 ml distilled water	300 ml ethanol + 300 ml distilled water
Carbohydrates	-	-	-
Proteins	-	-	-
Aminoacids	-	-	-
Glycosides	-	-	-
Alkaloids	+	+	+
Flavonoids	-	-	-
Tannins	-	-	-
Polyphenols	-	-	-
Diterpenoids	-	-	-
Steroids	-	-	-
Saponins	-	-	-
+ = Present - = Absent			

Table 2: anthelmintic activity of piperine isolated from black pepper using ethanol and hydroalcohol as solvents

Groups	Treatment	Concentration (mg/ml)	Time taken for Paralysis (min)	Time taken for Death (min)
1	Vehicle	-	-	-
2	Albendazole	20	42.28	71.34
Under reflux		2.5	105	190
		5	80	170
3	600ml 0f ethanol, (30min)	10	70	130
		25	45	75
		50	30	55
Maceration		2.5	98	198
		5	82	173
4	360ml ethanol+240 ml distilled water	10	75	145
		25	55	80
		50	33	70
5	300ml ethanol +3 00ml distilled water	2.5	100	205
		5	90	85
		10	78	160
		25	60	83
		50	33	75

5. Discussion on Results

From the results it was concluded that piperine isolated from black pepper using ethanol and hydroalchole as a solvent by under reflux and maceration showed anthelmintic activity in dose dependent manner.

The results also revealed that the piperine isolated using ethanol as a solvent by reflux took the less time to cause paralysis and death of the earthworm than those of piperine isolated from hydroalchole as a solvent by maceration process. From results the piperine as an anthelmintic activity has been confirmed as it displayed activity against the worm used in present study.

6. Conclusion

Helminthiasis is a parasitic disease that is concerned with infestation of gastro-intestinal tract of humans. Anthelmintics are those that kill or expel parasitic worms from the body. The present study aimed to evaluate the anthelmintic activity of piperine. The various concentrations of isolated piperine from black pepper by using ethanol and hydroalchole as solvent (100, 150, 200, 300, 500 mg/ml) respectively were screened for their anthelmintic activity by using Pheretima postuma and activities were comparable with the standard drug albendazole. Both shown the dose dependent anthelmintic activity.

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