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PROTECTIVE EFFECT OF *PISTACIA KHINJUK* ON *PHERETIMA POSTHUMA* AND *ASCARDIA GALLI*

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ABSTRACT

The present study is an attempt to explore the anthelmintic activity and comparative study of aqueous, ethanolic, hydroalcoholic extracts of *Pistacia khinjuk* . The various doses of aqueous, alcoholic and hydro-alcoholic extracts were evaluated for their anthelmintic activities on *Pheretima posthuma* and *Ascardia galli* . The extracts exhibited significant anthelmintic activity at a higher concentration of 100 mg/ml concentration . The activities are well comparable with the standard drug, Piperazine citrate. Hydro-alcoholic extract showed better anthelmintic activity in comparison to the alcoholic and aqueous extract of *Pistacia khinjuk.* This study suggests the vermifugal \ vermicidal activity of this plant extract against helminthes.

INTRODUCTION

Helminthiasis is more common in developing countries with poorer personal and enviormental hygine .[1] Helminthes are parasitc to humans and other animals and are derived from phyla, Platyhelminthes and Nemathelminthes.[2] In human body , they harm by depreving food , causing blood loss ,injury to organ , intestinal or lymphatic obstruction and by secreating toxins . [1] In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anaemia, eosinophilia, and pneumonia. Although the majority of infections due to worms are generally limited to tropical regions, they can occur to travellers who have visited those areas and some of them can develop in temperate climates. Parasitic diseases cause severe morbidity, including lymphatic filariasis (a cause of elephantiasis) onchocerciasis (river blindness), and schistosomiasis. These infections can affect most populations in endemic areas with major economic and social consequences.[3]

In the search for plant-based anthelmintics, extracts of different medicinal plants have been tested for action against flatworms and roundworms in vitro and in vivo and have been found to possess anthelmintic activity.[4]

Pistacia khinjuk (Family : Anacardiaceae) commonly known as kakadshingi.[5] It is found in Iran, Turkey, Baluchistan, Afghanistan, the Himalayan region (Kashmir to Nepal) and Myanmar at the elevation range up to 2400 meters .

In the essential oil obtained from the gum of *Pistacia khinjuk* , α-pinene (61.13%) was the principal constituent followed by myrcene (8.28%) , β-pinene (2.51%) *p*-cymene (2.50%) , 3-carene (1.36%) , linalool (2.76%) , and β-caryophyllene (1.95%) . Other minor

terpenes such as α-thujene, camphene , α-fenchene, sabinene , α-phallendrene , β-phallendrene , limonene , cineol , fenchone , borneol, and α-terpineol were also detected. Its leaves essential oil contained only monoterpene alcohols and was devoid of sesquiterpenes . The fruit essential oil contained 34 components of which *cis*-ocimene (24%) , α-pinene (17.9%), myrcene (14%) and *trans*-ocimene (8%) were the major constituents . Hydrocarbons (0.9%) , wax esters (1.3%), triglycerides (62.3%) , free fatty acids (4.9%), 1:3-diglycerides (15.6%) , 1:2-diglycerides (7.7%) , 2-monoglycerides (4.0%) and 1-monoglycerides (3.3%) , were found in *Pistacia khinjuk* lipids . Flavonoid glycosides such as quercetin-3-glucoside, quercetin-3-rutinoside , myricetin-3-glucoside, myricetin-3-galactoside and myricetin-3-rutinoside have been detected in *Pistacia khinjuk* .[6]

Ancient Greek physicians, such as Hippocrates, Dioscorides, Theophrastos , Galenos have recommended use of mastic gum from genus *Pistacia khinjuk* for gastrointestinal disorders like gastralgia, dyspepsia and peptic ulcer. Species of *Pistacia khinjuk* have been used in folk medicine as anti inflammatory, antipyretic, antibacterial, antiviral, in treatment of diarrhoea and throat infection.[7] It works as tonic and expectorant. It has been used in cough, phthisis, fever and asthma.[6]

The paper presents result of study designed to validate *Pistacia khinjuk* as anthelmintic against *Pheretima posthuma* and *Ascardia galli*.

MATERIAL AND METHODS

Plant materials

The plant material was procured from Shri Shail Medifarms Pvt. Ltd. Nagpur , Maharashtra , India . The plant was identified and authenticated by

botanists of Shri Shail Medifarms Pvt. Ltd. Nagpur, Maharashtra, India (voucher specimen no. SBMS/10/43) . Later, stored in airtight container and used for further successive extraction .

Preparation of extract *:*

Aqueous extract :

Powdered material of *Pistacia khinjuk* (100gm) was kept for maceration with 3000 ml of distilled water for 48 hrs. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation . The extract was dried and used as a powder.

Alcoholic extract :

Powdered material of *Pistacia khinjuk* (100gm) was kept for maceration with 3000 ml of ethanol for 48 hrs. 20%,30%,50% alcoholic extract was prepared . The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation. The extract was dried and used as a powder.

Hydro-alcoholic extract :

Powdered material of *Pistacia khinjuk* (100gm) was kept for maceration with 3000 ml of equal parts of distilled water and ethanol for 48 hrs .The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation .The extract was dried and used as a powder.

Experimental animals :

Adult earthworms (*Pheretima posthuma),* Roundworm (*Ascaridia galli)* were used to evaluate anthelmintic activity in *vitro*. *Pheretima posthuma* owing to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings were used for preliminary evaluation anthelmintic activity.[3][8][9][10][11] *Ascardia galli* were obtained from intestine of freshly slaughtered buffaloe. Infested intestines of buffaloes were collected

from the local slaughter house and washed with normal saline solution to remove all the faecal matter. These intestines were then dissected and worms were collected .

Preparation of standard solution and control :

Piperazine citrate standard drug was used of 25,50,100 mg/ml concentrations and distilled water as control .

Experimental procedure *:*

The worms were divided into seven groups with three subdivisions receiveing different concentrations of extract each of with six worms.

Group I: served as control and received only distilled water .

Group II: served as standard and received Piperazine citrate of 25, 50,100mg/ml concentrations .

Group III: received a aqueous extract of *Pistacia khinjuk* of 25,50,100 mg/ml concentrations .

Group IV: received a 20% alcoholic extract of *Pistacia khinjuk* of 25,50,100 mg/ml concentrations .

Group V: received 30% alcoholic extract of *Pistacia khinjuk* of 25,50,100 mg/ml concentrations .

Group VI: received 50% alcoholic extract *Pistacia khinjuk* of 25,50,100 mg/ml concentrations respectively .

Group VII: received hydroalcoholic (50:50) extract *Pistacia khinjuk* of 25,50,100 mg/ml concentrations respectively.

The time for paralysis and death of individual worm was observed and noted. Time for paralysis was recorded when no movement of any sort could observed ; time for death of worms was recorded when worms neither moved while shaken vigorously nor when dipped in warm water (50^{o}c) Potency is inversely proportional to

time taken for paralysis and/or death of worms.[3] [9][10][11]

Statistical analysis *:*

All the data are expressed as mean ± S.E.M.(standard error of mean) The Significance level was determined using the student's 't' test. A p value < was considered statistically significant.

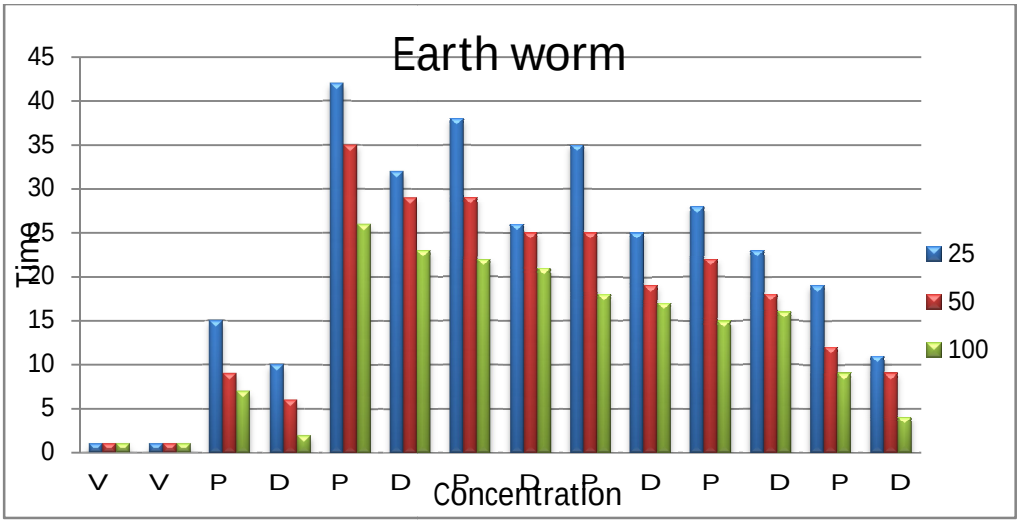
Result:

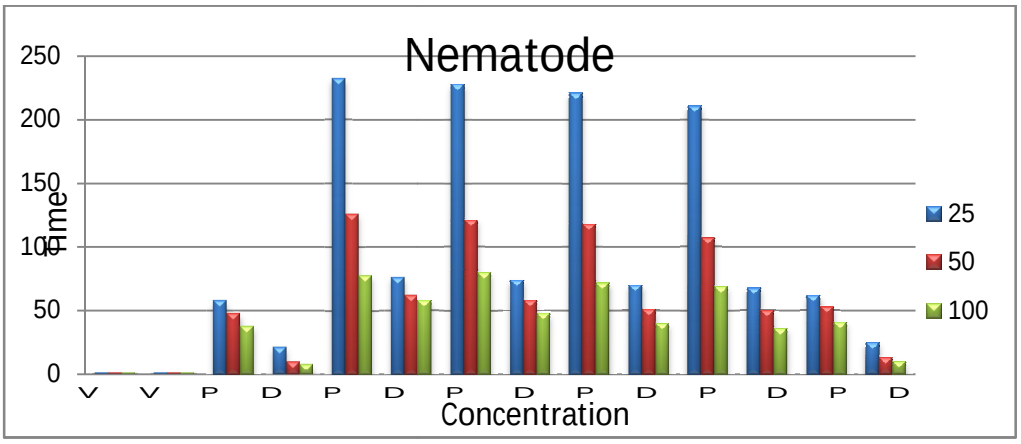
Table 1: *In vitro* anthelmintic activity *Pistacia khinjuk*.

Groups		Treatment	Conc. (mg /ml)	Paralysis Time (min.)		Death Time (min.)	
				EW	NT	EW	NT
I.	1	Vehicle	-	-	-	-	-
II.	2	Piperazine Citrate	25	15 ±0.6	10 ±1.2	58 ±0.7	21 ±3.8
	3		50	9.0 ±0.9	6.0±0.4	48 ±0.2	10 ±2.6
	4		**100**	**7.0** ±0.3	**2.0** ±0.9	**38** ±0.3	**8.0**±1.3
III.	5	Aqueous extract of *Pistacia khinjuk*.	25	42 ±3.4	32 ±2.7	232±9.4	76 ±3.4
	6		50	35 ±4.4	29 ±0.4	126±7.9	62 ±4.8
	7		**100**	**26** ±1.2	**23** ±0.8	**78** ±4.1	**58** ±2.6
IV.	8	20% Alcoholic extract of *Pistacia khinjuk*.	25	38 ±2.4	26 ±0.7	228±8.2	74 ±4.7
	9		50	29 ±3.1	25 ±1.3	121±3.7	58 ±1.8
	10		**100**	**22** ±1.2	**21** ±0.4	**80** ±4.6	**46** ±0.7
V.	11	30% Alcoholic extract of *Pistacia khinjuk*.	25	35 ±1.8	25 ±3.8	221±4.1	70 ±4.8
	12		50	25 ±1.2	19 ±0.8	118±5.3	51 ±0.8
	13		**100**	**18** ±0.4	**17** ±1.4	**72** ±1.4	**40** ±1.6
VI.	14	50% Alcoholic extract of *Pistacia khinjuk*.	25	28 ±3.1	23 ±1.3	211±4.7	68 ±1.4
	15		50	22 ±5.1	18 ±0.4	107±5.6	50 ±1.1
	16		**100**	**15** ±2.1	**16** ±0.7	**69** ±2.1	**36** ±0.4

VII.	17	Hydro-alcoholic extract of *Pistacia khinjuk*.	25	19 ±0.5	11 ±0.8	62 ±0.5	25 ±2.5
	18		50	12 ±0.7	9.0±0.2	53 ±0.1	13 ±2.1
	19		**100**	**9.0**±0.1	**4.0**±0.6	**41** ±0.2	**10** ±1.4

min. = Minutes; **EW**= Earth Worm; NT= Nematode; **Conc**. = Concentration.





V – Vehicle, P – Paralysis, D – Death.

Concentration – ● 25mg/ml ● 50mg/ml ● 100mg/ml

Figure 1&2: Anthelmintic activitiy of *Pistacia khinjuk* extracts on paralysis and death of earthworm and nematode.

Discussion :

Helminthic infections of the gastrointestinal tract of human beings and animals have been recognized to have adverse effects on health standards with a consequent lowering of resistance to other disease . [3]

Chemotherapeutic agents available for treatment of helminthe infection act mainly through three different mechanisms, viz, by disruption of the neuromuscular physiology, by blocking the energy metabolism, and by disturbing the highly efficient reproductive system of the parasites. Several important anthelmintics cause paralysis of helminth parasites by disrupting one or the other aspect of their neuromuscular system . [4]

The result depicts the time taken for paralysis and death of worms after treating with test substance. The activity was comparable with that of standard drug Piperazine citrate. The result of study showed that hydroalcoholic (50:50) extract *Pistacia khinjuk* has significant anthelmintic activity at 100 mg/ml extract as compared to 25 and 50 mg/ml extracts . The standard Piperazine citrate showed enhanced anthelmintic activity. The extract showed activity which was almost equal in effectiveness to standard Piperazine citrate. The difference in time taken for induction of paralysis in both Piperazine citrate and hydroalcoholic extract of *Pistacia khinjuk* was significant or almost same. Hydro-alcoholic extract showed better anthelmintic activity in comparison to the alcoholic and aqueous extract of *Pistacia khinjuk.*

Piperazine blocks the response of the worm muscle to ACh , presumbaly by causing hyperpolarisation of nerve endings, resulting in flaccid paralysis of worms .While worm is paralysed ,it is dislodged from the intestinal lumen and expelled live from body by normal intestinal peristalsis .[12]

The mechanism action of *Pistacia khinjuk* is not yet fully understood, but the anthelmintic activity could be attributed to bioactive compounds of *Pistacia khinjuk* jointly or separately. The phytochemical analysis of *Pistacia khinjuk* revealed the presence of terpenoids , essential oils , flavonoids, and lipids . In this study we have evaluated the effect of *Pistacia khinjuk* extracts on and *Pheretima posthuma* , *Ascardia galli*. Hydroalcoholic extract showed significant vermicidal activity.

In conclusion, results from this current study indicate that *Pistacia khinjuk* has the potential as an anthelmintic.

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