WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 214-223. Review Article ISSN 2277– 7105

A REVIEW ON ANTHELMENTIC ACTIVITY OF *CITRUS AURANTIFOLIA LINN* FRUITS EXTRACT

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Article Received on
26 July 2016,

Revised on 16 August 2016,
Accepted on 06 Sep. 2016

DOI: 10.20959/wjpr201610-7070

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ABSTRACT

Objective: The present study was done with the aim to evaluate the anthelmintic activity of aqueous extracts of fruits Citrus aurantifolia Linn belonging to family Rutaceae. **Method:** The aqueous extracts of fruits Citrus aurantifolia was tested using adult earthworm Pheritima posthuma against Piperazine citrate (10 mg/ml) and distilled water were included in the assay as standard reference drug and control, respectively. The time to achieve paralysis of the worms was determined. **Result:** aqueous extracts of fruits Citrus aurantifolia produced a potent anthelminthic activity against the P. posthuma when compared with reference standards ($p < 0.001$). **Conclusion:** This study shows aqueous extracts of fruits Citrus aurantifolia has paralytic effect on Indian earthworms.

KEYWORDS: Citrus aurantifolia, Pheretima postuma, Piperazine citrate, Anthelminthic activity.

INTRODUCTION

Helminthes infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminthes are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anemia, eosinophilia and pneumonia.[1] Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas.[2] The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases.[3] Treatment with an antihelminthic drug kills worms whose genotype renders them susceptible to the drug.

Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs. Intestinal worm infections in general are more easily treated than those in other locations in the body.[4] Because the worms need not be killed by the drug and the drug need not be absorbed when given by mouth; there is usually a wider margin of safety than with drugs for worm infections in other sites. Indiscriminate use of synthetic anthelmintics can lead to resistance of parasites.[5] Food supplements like papaya (Carica papaya), cinnamon (Cinnamomum camphora, C.zeylanicum), turmeric(Curcuma longa), asafoetida (Ferula foetida), long pepper (Piper longum), blackpepper (Piper nigrum), carrot (Daucus carota), saffron(Crocus sativus), Moringa (Moringaptery gosperma), bitter guard (Momordica charantia) and fresh juices of pine apple have anthelmintic property.[6] Hence there is an increasing demand towards natural anthelmintics and to prevent resistance. Citrus aurantifolia contains terpineol (9.41%) and terpinene (6.26%) limonene (32.6%), terpineol (12.5%) and pinene (6.3%), 5,7-dimethoxycoumarin (6.6%), and bergapten (2.9%), terpinolene, limonene, fenchene, camphene, terpinene. And also useful To prevent scurvy. Lime extracts and lime essential oils are frequently used in perfumes, cleaning products, and aromatherapy. In India, the lime is used in Tantra for removing evil spirits. It is also combined with Indian chillies to make a protective charm to repel the evil eye. Furthermore, it was believed that hanging limes over sick people cured them of the illness by repelling evil spirits lurking inside the body. In cooking, lime is valued both for the acidity of its juice and the floral aroma of its zest. It is a common ingredient in authentic Mexican, Vietnamese and Thai dishes. It is also used for its pickling properties in ceviche. Some guacamole recipes call for lime juice. Lime is an essential ingredient in several highball cocktails, often based on gin, such as gin and tonic, the gimlet and the Rickey. Freshly squeezed lime juice is also considered a key ingredient in margaritas, although sometimes lemon juice is substituted.

HELMINTHIASIS

Helminthiasis or worm infection is one of the most prevalent diseases. Many worms are parasitic in humans and causes serious complication. It is estimated that one fourth of the world population may be infected by worms. In Helminthiasis this organism multiply outside of the definitive host and have the unique ability to evade host immune defenses, for reasons that are not fully understood. Helminthiasis tends to be chronic, possibly lasting an entire lifetime of the host.

Infected host humans are divided in to two categories or phyla.

1. Platy helminthes (flateworms):-

In which a. Cestodes (tapeworms), b. Trematode (flukes) are included.

2. Nematodes (roundworms):-

In which a. Roundworm, b. Hookwor, c. Pinworm, d. Whipworm are included.

Drugs are use in Helminthiasis

1. Benzimidazoles:-

Ex-mebendazole, thiabendazole, albendazole, oxybendazole, parbendazole.

2. Diethyl carbamazine.

3. Ivermectine.

4. Praziquantel.[17]

Diseases caused by helminthes parasites in continue to be a major productivity constraint, especially in small ruminants in the tropics and Subtropics. In the developing world, the greatest impact of parasitic diseases is indirect and potential productivity losses. Infections by gastrointestinal helminthes parasite of livestock are among the most common and economically important diseases of grazing livestock. Adulteration of anthelmentics has been found to be common practice. Illiteracy and unfamiliarity with synthetic anthelmentics, resulting in incorrect usage, are also a problem leading to the same consequence. Moreover, these drugs are relatively expensive. As a consequence of these problems and difficulties, pastoralists and small holder farmer have continued to use indigenous plants as livestock dewormers. Considerable research has shown that some plants not only affect the nutrition of animals, but also have antiparasitic effects. For Example, plants that contain condensed tannins, a class of phenolic secondary metabolite, have these effects.

INTRODUCTION TO PLANT

Plant: Citrus aurantifoia

Synonyms: West Indian Lime, Bartender's Lime, Omani Lime or Mexican Lime dayap and bilolo.

Parts used: Seeds, Leaves, Fruits and Bark

CHEMICAL COMPOSITION

terpineol (9.41%) and terpinene (6.26%) limonene (32.6%), terpineol (12.5%) and pinene (6.3%), 5,7-dimethoxycoumarin (6.6%) and bergapten (2.9%), terpinolene, limonene, fenchene, camphene, terpinene.

USES

To prevent scurvy. Lime extracts and lime essential oils are frequently used in perfumes, cleaning products and aromatherapy. In India, the lime is used in Tantra for removing evil spirits. It is also combined with Indian chillies to make a protective charm to repel the evil eye. Furthermore, it was believed that hanging limes over sick people cured them of the illness by repelling evil spirits lurking inside the body. In cooking, lime is valued both for the acidity of its juice and the floral aroma of its zest. It is a common ingredient in authentic Mexican, Vietnamese and Thai dishes. It is also used for its pickling properties in ceviche. Some guacamole recipes call for lime juice. Lime is an essential ingredient in several highball cocktails, often based on gin, such as gin and tonic, the gimlet and the Rickey. Freshly squeezed lime juice is also considered a key ingredient in margaritas, although sometimes lemon juice is substituted.

MATERIALS AND METHODS

Materials

The fruits of Citrus aurantifolia have been collected from the local area of Nandurbar (Maharashtra).

Preparation of extract

Collected fruits were dried and crushed to a coarse powder and subjected to maceration with water. Macerate was dried over anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by using rotary evaporator. The aqueous extract was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds.[16]

Worms Collection and Authentication

Indian earthworm *Pheritima posthuma* (Annelida) were collected from the water logged areas of soils Indian earthworms.

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anhydrous sodium sulphate and solvent was removed in vacuum at 40°C by using rotary evaporator. The aqueous extract was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds.[7] Indian earthworm Pheritima posthuma (Annelida) were collected from the water logged areas of soils different concentration of crude aqueous extract (25, 50 and 100 mg/ml in distilled water) were prepared and 6 worms of same type were placed in it. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50^0C). Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control.

DRUGS AND CHEMICALS

Piperazine citrate (Noel, Mumbai) and distilled water were used as reference standards. Normal saline were used as control.

Methods

Ash value[18]

1. Total Ash value

Weight accuratily of subtance and placed in a tared dish. Evaporate it at low temperature. It possible untill the solvent is removed, heat it an waterbath. Untill the residue is apparently dry. Transfer to oven and dry it at constant temperature at 105^oc, otherwise stated in monograph. Hygroscopic nature of certain residue it may be necessary to use dishes provided with well fitting coverrs and cool in desicator.

2. Acid Insoluble Ash Value

Boil the ash with 25ml of 2m HCl for 5 min. Collect the insoluble matter in gooch crucible or ashless filter value. wash it with hot water. Ignite and cool in desicator and weight and calculate the percentage acid insoluble ash to air dried drug.

3. Water soluble ash value

Boil ash for 5 min. with25 ml water. collect the in soluble matter in gooch filter or ash less filter paper. wash it with hot water. Ignite for 15 min. at temperature not exceed 450^oC. subtract the insoluble weight from at the ash the different in weight. Represent the water soluble ash and calculate percentage of water soluble ash.

4. Determination of Loss On Drying[19]

Weigh about 1.5 gm of the powdered drug into a weighed flat and thin porcelain dish. Dry in oven at 100°c. Cool it desicator and watch the losses in weight is usually recorded as moisture.

Table No: 1 Pharmacognistic study of *Citrus aurantifolia.*

Sr No.	Content	Values
1.	Total ash value	5%
2.	Water soluble ash value	2.3%
3.	Acid insoluble ash value	2%
4.	Loss On Drying	6.4%

Chemical Test[20]

1. Test for carbohydrate

Sr.no	Test	Inference
1.	Molish test:- extract α-napthol in alcohol+Con.H_2SO_4.	+
2.	Fehllingtest:extract+FehlingA+B.boil on water bath for 5min.	+
3.	Benedicts test:extract+benedict reagent.	+

2. Test for Protein

Sr.no	Test	Inference
1.	Biueret test:extract+4%NaOH+1%$CuSO_4$	+
2.	Millions test:extract+Millions reagent.	+

3. Test for Tannins

Sr.No.	Test	Inference
1.	Extract + 5% $Fecl_3$	+
2.	Extract + Dil. HNO_3	+
3.	Extract + Dil. Iodine	+

3. Test for Glycoside

Sr.No.	Test	Inference
1.	Baljet test:extract+Na picrate	+
2.	Legal test:exract+1ml pyridine+1ml Na nitropruside.	+
3.	Killer killani test:extract+gla.acetic acid+1 drop 5%$FeCl_3$ +conc.H_2SO_4.	+

5. Test for alkaloid

Sr.No.	Test	Inference
1.	Dragondorffstest:Extract+ dragondorffs reagent	+
2.	Mayers test: Extract+ mayers reagent	+
3.	Hagers test: Extract+hagers reagent	+

6. Test For Flavanoids

Sr.No.	Test	Inference
1.	ShinodaTest: extract+5ml 95% ethanol.+con.HCL+0.5gm Mg-turnings.	+

7. Test for Steroids

Sr.No.	Test	Inference
1.	Libermanns Reaction:-Extract+acetic anhydride heat, boil+con. h-H_2SO_4.	+

9. Test for Saponins

Sr.No.	Test	Inference
1.	Foam Test:-Dried poweder +water shake	+

ANTHELMINTIC INVESTIGATION

The anthelminthic activity was evaluated in adult earthworm (Pheretima posthuma) due to its anatomical and physiological resemblance with the intestinal round worm parasites of human beings 50 ml. of formulation containing different concentration of crude aqueous extract (25, 50 and 100 mg/ml in distilled water) were prepared and 6 worms of same type were placed in it.

Observations were made for the time of paralysis of individual worms. Paralysis assumed to occur when the worms did not revive even in normal saline. Observations were made for the time taken to paralyze 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.[8]

STATISTICAL ANALYSIS

The data presented as Mean ± SEM. The activities of both the Leaves extracts were compared with the control. All the extracts showed significantly higher duration of paralysis and death. Values of $P<0.001$ were considered statistically significant.[9]

RESULTS AND DISCUSSION

Phytochemical screening of both extract of fruits of Citrus aurantifolia revealed the presence of alkaloids, saponins, flavonoids and tannins. As shown in Table 1, aqueous extract exhibited anthelmentic activity in dose dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml, 50 mg/ml, 25 mg/ml concentration for Pheritima posthuma worms. The aqueous extract of fruits of Citrus aurantifolia caused paralysis is 8

min., 32 min., 224 min. and time of death is 20 min., 156 min., 289 min. respectively against Pheritima posthuma. The reference drug Piperazine citrate showed the paralysis at 22 min. and time of death at 100 mg conc. 49 min. respectively.

Piperazine citrate by increasing chloride ion conductance in worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis.[10] The aqueous extract of leaves of Vitex negundo not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts revealed the presence of tannins among the other chemical constituent within them. Tannins were shown to produce anthelmintic activities.[11] Chemically tannins are polyphenolic compounds.[12] Some synthetic phenolic anthelmintics e.g. niclosamide, oxyclozanide, bithionol etc., are reported to interfere with energy generation in helminth parasites by uncoupling oxidative phosphorylation.[13] It is possible that tannins contained in the aqueous extract of leaves of Vitex negundo produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tracts of host animal[14] or glycoprotein on the cuticle of the parasite[15] and may cause death.

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in developing countries, including in India. It is in this context that the people consumed several plants or plant derived preparation to cure helminthic infections.[16] The origin of many effective drugs has been found in the traditional medicines practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their proclaimed anthelmintic efficacy.

Table: 2 Anthelmintic activity of aqueous extract of Citrus aurantifolia Linn. fruits

	Concentration in mg/ml	**Time taken for Paralysis (P) and Death (D) of worms in minute**	
		Pheritima posthuma	
		P	D
Control (in normal saline)	-	-	-
Aqueous extract	25	0.34	±0.78
Aqueous extract	50	32.22±0.21	1.96±0.29
Aqueous extract	100	6.7±0.12***	19.90±0.21***
Piperazine citrate	10	21.56±0.34	48.70±0.78

All values are from referene MEAN±SEM, N=6 in each group are significantly different from reference standard (Piperizine citrate).
*P<0.05,**P<0.005,***P<0.001.

CONCLUSION

In Conclusion, the traditional use of plants Citrus aurantifolia an anthelmintic have been confirmed, it would be interesting to isolate the possible phytoconstituents, which may be responsible for the anthelmintic activity and to reveal the mechanism (s) of actions.

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