International Journal of Drug Development & Research
| Jan-March 2011 | Vol. 3 | Issue 1 | ISSN 0975-9344 |
Available online http://www.ijddr.in
Covered in Official Product of Elsevier, The Netherlands

Full Length Research Paper
Covered in Official Product of Elsevier, The Netherlands

Investigation of in Vitro Anthelmintic activity of *Cinnamomum Camphor Leaves*

HAQUE RABIUL1, MONDAL SUBHASISH2, GHOSH PARAG2,
*1Department of Biotechnology, Institute of Technology & Marine Engineering, D.H.Road, West Bengal,
2 Department of Pharmaceutical Technology, Jadavpur university,West Bengal, India.

Abstract
The aqueous extract of Cinnamomum camphorLeaves was investigated for anthelmintic activity using earthworms(Pheretima posthuma), tapeworms (Raillietina spiralis) and roundworms (Ascaridia galli). Various concentrations (10-70 mg/ml) of plant extract were tested in the bioassay. Piperazine citrate (10 mg/ml) was used as reference standard drug whereas distilled water as control.Determination of paralysis time and death time of the worms were recorded. Extract exhibited significant anthelmintic activity at the concentration of 50 mg/ml. The result shows that aqueous extract possesses vermicidal activity and found to be effective as an anthelmintic. Therefore, the anthelmintic activity of the aqueous extract of Cinnamomum camphorLeaves has been reported.

Introduction

Infections with helminth are among the most widespread infections in humans and other domestic animals affecting a large number of world population. The majority of these infections due to worms are generally restricted mainly to the tropical regions and the occurance is accelerated due to unhygienic lifestyle and poverty also resulting in the development of symtomps like anaemia, eosinophilia and pneumonia1. Parasitic diseases cause ruthless morbidity affecting principally in population.

*Corresponding author, Mailing address:
Prof (Dr) Rabiul Haque,
Institute of Technology & Marine Engineering.
South 24 Parganas,
West Bengal,
India. Phone-+91 98317-85874.
E-mail- ruuubil_haque@yahoo.com

Key words:
Cinnamomum camphor Leaves, , vermicidal, anthelmintic activity

How to Cite this Paper:
Haque Rabiu, Mondal Subhasish, Ghosh Parag, "Investigation of in Vitro Anthelmintic activity of *Cinnamomum Camphor Leaves*", Int. J. Drug Dev. & Res., Jan-March 2011, 3(1): **295-300**

Article History:----------
Date of Submission: 23-10-2010
Date of Acceptance: 25-01-2011
Conflict of Interest: NIL
Source of Support: NONE

Introduction:

Synonym: Camphor tree, Gum camphor, camphor laurel.[1,2,3,45,] Botanical name: *Cinnamomum camphor*Scientific Classification:Kingdom- Planate Subkingdom- Tracheobionta, Superdivision- Spermatophyta Division- Magnoliophyta, Class- Magnoliopsida ,Subclass –Magnoliidae, Order- Laurales Family- Lauraceae, Genus- Cinnamomum

,Species- Cinnamomum verum, Cinnamon is from a tropical ever green tree of the laurel family growing up to 7m(56feet) in itswild state. It has thick scabrous bark, strong branches, young shoots, speckled greeny orange. The bark is smooth and yellowish. The leaves are ovate, petiolate,deeply veined leaves that are dark green on top, lightgreen underneath. They become leathery when matureupper side shiny green, under side lighter. When bruished they smell spicy and have a hot taste. The flowers are yellowish white with disaggreable odour that bears dark berries. The fruit is an oval berry like an acron in its receptical is bluish when ripe with white spots on it, bigger than a blackberry.Chemical constituents: Cinnamaldehyde, mannitol, coumarins, gum, tannins, essential oils (aldehyde,eugenol, pinene).

[www.globalresearchonline.net] It is one of the major components in Ayurvedic medicine, which has been practiced in India since many centuries. The is an ancient Indian cure-all due to its antibacterial, antifungal, antiviral, antihistamine and antiseptic properties.The *Cinnamomum camphor* leaves, flowers, seeds, roots, bark and fruits are utilized to treat inflammation, infections, skin diseases. Antihelminthics or anthelmintics are drugs that expelparasitic worms (helminthes) from the body by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing). The worms causing heliminthiasis are Round worms, Hook worms, Tape worms, Pin worms, Whip worms e.t.c.

Cinnamomum camphora (commonly known as **Camphor tree**, **Camphorwood** or **camphor laurel**) is a large evergreen tree that grows up to 20–30 metres tall. The leaves have a glossy, waxy appearance and smell of camphor when crushed. In spring it produces bright green foliage with masses of small white flowers. It produces clusters of black berry-like fruit around one centimetre in diameter. It has a pale bark that is very rough and fissured vertically.Camphor is a white crystalline substance, obtained from the tree *Cinnamomum camphora*.Camphor has been[6,7,8,9] used for many centuries as a culinary spice, a component of incense, and as a medicine. Camphor is also an insect repellent and a flea-killing substance.*Cinnamomum camphora* is native to Taiwan, southern Japan, southeast China and Indochina, where it is also cultivated for camphor and timber production. The production and shipment of camphor, in a solid, waxy form, was a major industry in Taiwan prior to and during the Japanese colonial era (1895–1945). It was used medicinally and was also an important ingredient in the production of smokeless gunpowder and celluloid. Primitive stills were set up in the mountainous areas in which the tree is usually found. The wood was chipped; these chips were steamed in a retort, allowing the camphor to crystallize on the inside of a crystallization box, after the vapour had passed through a cooling chamber. It was then scraped off and packed out to government-run factories for processing and sale. Camphor was one of the most lucrative of several important government monopolies under the Japanese.In the ancient and medieval Middle East and Europe, camphor was used as ingredient for sweets but it is now mainly used for medicinal purposes. For example, camphor was used as a flavoring in confections resembling ice cream in China during the Tang dynasty (AD 618–907). An anonymous Andalusian cookbook of the 13th century contains a recipe for meat with apples, which is flavored with camphor and musk.[1] A 13th century recipe for "Honeyed Dates" is also flavored with Camphor.[2] By the time of the Renaissance, camphor as a culinary ingredient had fallen into disuse in Europe.Today, camphor is widely used in cooking (mainly for dessert dishes such as kheer or paal paayasam) in India where it is known as *pachha karpooram* (literally meaning "green camphor"). It is widely

available at Indian grocery stores and is labeled as "edible camphor". In Hindu poojas and ceremonies, camphor is burned in a ceremonial spoon or plates for performing aarti. This type of camphor is also sold at Indian grocery stores but it is not suitable for cooking.The twigs and leaves of the camphor plant are used in the smoking and preparation of Zhangcha duck, a typical banquet and celebratory dish in Szechuan cuisine

In India's ancient Ayurvedic Medical texts it is explained that every part of the *: Cinnamomum camphor* tree has health promoting benefits. What is clear from the above information is that the general population of India for, over 5000 years, has used *: Cinnamomum camphor* safely and effectively. It is also called Holy Tree (*: Cinnamomum camphor* In ancient times neem was the most celebrated medicinal plant of India and found mention in a number of Puranic texts like the Atharava Veda, Upanivahod, Amarkosha and Ghrysutra. They all dealt with the outstanding qualities of the *Cinnamomum camphor* tree as a source of medicine pesticide.. Today, neem is once again steadily becoming an agro-scientific celebrity. It has figured as the priority in seminars and serious agricultural workshops all over the world. Modern western medicine is finally discovering what the ancient Indians have known for thousands of years: that the *: Cinnamomum camphor* tree has superb pharmaceutical and pesticide controlling qualities. Its effectiveness, availability and safety have made agro-scientists promote cultivation of*: Cinnamomum camphor* forests. Camphor trees grow in full sun to partial shade. They tolerate clay, loam, sand, slightly alkaline to acidic soils, and drought.[10,11,12,,13,] They need to be well drained or they may suffer from root rot , Camphor laurel contains volatile chemical compounds in all plant parts, and the wood and leaves are steam distilled for the essential oils. Camphor laurel has six different chemical variants called chemotypes, which are camphor, linalool, 1,8-cineole, nerolidol, safrole, or borneol. In China field workers avoid mixing chemotypes when harvesting by their odour.[8][9] The cineole fraction of camphor laurel is used in China to manufacture fake "Eucalyptus oil".[10]

The chemical variants (or chemotypes) seem dependent upon the country of origin of the tree. The tree is native to China, Japan, and Taiwan. It has been introduced to the other countries where it has been found, and the chemical variants are identifiable by country. i.e., *Cinnamomum camphora* grown in Taiwan and Japan, (often commonly called "Ho Wood") is normally very high in Linalool, often between 80 and 85%. In India and Sri Lanka the high camphor variety/chemotype remains dominant. The *Cinnamomum camphora* grown in Madagascar, on the other hand, is high in 1,8 Cineole (averaging between 40 and 50%. The essential oil from the Madagascar trees is commercially known as Ravintsara.[11]

Materials and methods

Plant Materials

The *Cinnamomum camphora* tree was collected from the nursery (Amtala) south 24 paraganas district, West Bengal, India. The plant material was taxonomically identified by the taxonomists of Botanical Survey of India, Kolkata. A voucher specimen has been preserved in our laboratory for future reference. The plant material was dried in shade, pulverized, passed through sieve no. 40 and stored in air tight container and used for further extraction.

Preparation of extract

Aqueous extract (Maceration method)

Mature green leaves,pulverized,root, wood are collected and allowed to dry partially. These dried leaves , root,wood are then crushed and powdered. The crushed leaves are then subjected to either

aqueous or organic solvent to get a concentrated extract. For making neem leaf extract, certain extraction process utilizes carbon dioxide at critical temperatures and pressures to extract the active ingredients of the leaf, the usual high temperatures or harsh chemicals are done away with, resulting in a better concentrated and potent extract.

The plant leaves were dried in shade, pulverized and then powdered material of *Cinnamomum camphora* powder(500gm) were kept for maceration with 1000 ml of double distilled water for 24 hours. The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder. The percentage yield of extract was found to be about 4%.

Experimental Animals

Adult earthworms (*Pheretima posthuma),* Roundworm (*Ascaridia galli) and* Tapeworms (*Raillietina spiralis)* were used to evaluate anthelmintic activity in *vitro*. Earthworms were collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol. Roundworms and tapeworms were obtained from intestine of freshly slaughtered fowls. Infested intestines of fowls 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 double distilled water as control.[13,14,15,16] . This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments. Observations were made for the time taken for paralysis was noted when no movement of any sort could be observed except when the wormsand worms were collected and kept in normal saline solution.

The average size of round worm was 5-7 cm and average size of tapeworm was 6-8 cm. Earthworm and helminths were identified in Dept. of Zoology, Vivekananda College,Thakurpukur, Kolkata.

Drugs & Chemicals

Piperazine citrate (Glaxo Smithkline) was used as standard anthelmintic during the experimental protocol.

Anthelmintic activity

The anthelmintic assay was carried out as per the method of Ajaiyeoba et al 4 . The assay was performed in *vitro* using adult earthworm (*Pheretima posthuma)* as it is having anatomical and physiological resemblance with the intestinal round worm parasites of human beings for preliminary evaluation of anthelmintic activity [17,18,19]. Use of *Ascaridia galli* and *Raillietina* species as a suitable model for screening of anthelmintic drug was advocated earlier [20,21].

Test samples of the extract was prepared at the concentrations, 10, 20,30,40 , 50,60,70, mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard was advocated earlier 20,21.Test samples of the extract was prepared at the concentrations, 10, 20,30,40 , 50, 60 and 70 mg/ml in distilled water and six worms i.e. *Pheretima posthuma, Ascaridia galli* and *Raillietina spiralis* of approximately equal size (same type) were placed in each nine cm Petri dish containing 25 ml of above test solution of extracts. Piperazine citrate (10 mg /ml) was used as reference standard and double distilled water as control [14,15,16,17] . This procedure was adopted for all three different types of worms. All the test solution and standard drug solution were prepared freshly before starting the experiments.

Observations were made for the time taken for[22,23,24,25,26,27,,28] paralysis was noted when no movement of any sort could be observed except when the wormsTime for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50° C).

All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

Table – 1: Anthelmintic activity of *Cinnamomum camphora* aqueous extract

Each value represents mean ± SEM (N=6)

	GROUP		Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)	Time taken for paralysis (P) in min. (Mean & SEM)	Time taken for death (D) in min. (Mean & SEM)
01	Control (Water Only	.....	.....	.....	.....	.....	.....	.....
02	*Cinnamomum camphora*	10 20 30 40 50 60 70	32±0.33 25±0.25 19±0.55 17±0.38 17±0.11 22±1.17 19±0.56	53±0.23 46±0.27 30±0.64 31±0.72 32±0.25 62±0.77 45±0.28	28±0.37 23±0.78 14±0.29 14±0.55 13±0.32 13±1.58 24±0.77	55±0.57 52±0.43 38±0.56 39±1.25 39±1.101 38±1.19 38±0.28	34±1.65 24±0.22 20±1.67 19±0.59 19±0.15 26±0.56 26±0.53	61±1.45 47±0.54 40±1.18 41±0.50 41±0.11 53±0.14 49±0.17
03	Piperazine citrate	10	22±1.10	60±0.75	12±1.50	38±1.10	24±0.50	52±0.10

Each value represents mean ± SEM (N=6)

RESULTS AND DISCUSSIONS

From our observations, higher concentration of extract produced paralytic effect much earlier and the time taken for death was shorter for all types of worms. Aqueous extract of *Cinnamomum camphora* exhibited anthelmintic activity in dose-dependent manner showing maximum efficacy at 50 mg/ ml concentration for all three types of worms. Our plant extract exhibited more potent activity at lowest concentration (10 mg/ml) against (roundworm) *Ascaridia galli*. Anthelmintic activity of the extract was compared with the standard drug Piperazine citrate (Table.1). From the above results, we can conclude that *N*eem which is used traditionally to treat intestinal worm infections, exihibited significant anthelmintic activity. Therefore, further study must be carried out so that the general people can get actual benefit from this important medicinal plant.

Acknowledgments

We wish to thank the Director Dr Professor T.K Basu of my institute The Institute of Technology & Marine Engineering for his moral support and blessings and I also express my heartful thanks to him for motivating me with strength and spirit.

REFERENCES:

1) "An Anonymous Andalusian Cookbook of the 13th century" Translated Charles Perry, taken from Cariadoc¹s Miscellany
2) "In A Caliph's Kitchen" by David Waines
3) Noxious weed declaration for NSW
4) "Plants Profile: *Cinnamomum camphora*". *Natural Resources Conservation Service*. United States Department of Agriculture. http://plants.usda.gov/java/profile? symbol = CICA. Retrieved 12 April 2010.
5) Forest Starr, Kim Starr, and Lloyd Loope (January 2003). "*Cinnamomum camphora*". *United States Geological Survey: Biological Resources Division*. Hawaiian Ecosystems at Risk project. http://www.hear.org/Pier/pdf/pohreports/cinnamomum_camphora.pdf. Retrieved 12 April 2010. USDA Hardiness Zone
6) E.F. Gilman & D.G. Watson Fact Sheet ST-167 Cinnamomum Camphora Nov. 1993
7) Hirota, N. and Hiroi, M., 1967. 'The later studies on the camphor tree, on the leaf oil of each practical form and its utilisation', *Perfumery and Essential Oil Record* 58, 364-367.
8) Lawrence, B. M., 1995. 'Progress in essential oils', *Perfumer and Flavorist*, 20, 29-41.
9) Ashurst, P.R., *Food Flavorings*, 1999
10) Behra, Burfield, www.cropwatch.org/Ravensara-Ravintsara%20biblio%20v1.01.pdf
11) *Lal J, Chandra S, Raviprakash V, Sabir M. In vitro anthelmintic action of some indigenous medicinal*
12) *plants on Ascardia galli worms. Indian J Physiol Pharmacology 1976; 20: 64-68.*
13) *Mali RG, Shailaja Mahajan, Patil KS. Anthelmintic activity of root bark of Capparis spinosa. Indian J Nat Prod. 2005; 21: 50-51.*
14) *Mali RG, Wadekar RR. In Vitro anthelmintic activity of Baliospermum montanum Muell. Arg roots. Indian J Pharm Sci. 2008; Jan-Feb: 131-133.*
15) *Gbolade AA, Adeyemi AA. Investigation of in vitro anthelmintic activities of Pycnanthus angeolensis and Sphenocentrum jollyanum. Fitoterapia 2008; 79: 200-222.*
16) *Vigar Z. Atlas of Medical Parasitology. 2nd ed. P. G. Publishing House Singapore 1984. p. 242.*
17) *Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of Evolvulus alsinoides Linn. For anthelmintic and antimicrobial activities, J Nat Rem. 2002; 2: 182- 185.*
18) *Shivkumar YM, Kumar VL. Anthelmintic activity of latex of Calotropis procera. Pharma Biol. 2003; 41: 263-265.*
19) *Kaushik RK, Katiyar JC, Sen AB. Studies on th mode of action of anthelmintics with Ascardia galli as a test parasite. Indian J Med Res. 1974; 64: 1367-75.*
20) *Yadav AK, Temjenmongla. Anthelmintic activity of Gynura angulosa against Trichinella spiralis infections in mice. Pharmacologyonline 2006; 2: 299-306*
21) *Bundy D A. Immunoepidemiology of intestinalhelminthicinfection I: The global burden of intestinal nematode disease.Trans Royal Soc Trop Med Hyg 1994; 8: 259-261.*
22) *Tagbota S, Townson S. Antiparasitic properties ofn medicinal and other naturally occurring products, Adv Parasitol 2001; 50:199-205.*
23) *Sondhi SM., Shahu R., Magan Archana. Indian Drugs 1994; 31(7): 317-320.*
24) *Ajaiyeoba EO, Onocha PA, Olarenwaju OT. In vitro anthelmintic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm Biol. 2001; 39: 217- 20.*
25) *Vigar Z. Atlas of Medical Parasitology. 2nd ed. P. G. Publishing House Singapore 1984. p. 242.*
26) *Dash GK, Suresh P, Kar DM, Ganpaty S, Panda SB. Evaluation of Evolvulus alsinoides Linn. For anthelmintic and antimicrobial activities, J Nat Rem. 2002; 2: 182- 185.*
27) *Shivkumar YM, Kumar VL. Anthelmintic activity of latex of Calotropis procera. Pharma Biol. 2003; 41: 263-265.*