

In vitro Anthelmintic Activity Of *Wrightia tinctoria*

Rajalakshmi G.R.^{1*}, Jyoti Harindran²

¹Karpagam University, Karpagam, Coimbatore, Tamil Nadu-641021, India.

²University College of Pharmaceutical Sciences, Mahatma Gandhi University, Rubber Board (P.O), Kottayam, Kerala, India.

*Corres.Author : sijuellickal@rediffmail.com

Abstract: Alcoholic and aqueous extracts from leaves of *Wrightia tinctoria* were investigated for their anthelmintic activity against *Raillietina spiralis* and *Ascaridia galli*. The three concentration (10, 25, 50mg/ml) of extract were studied in activity which involved the determination of time of paralysis and time of death of the worm. The extract exhibited significant dose dependent anthelmintic activity. Piperazine citrate was included as standard reference and distilled water as control.

Key Words: *Wrightia tinctoria*, Anthelmintic activity, *Raillietina spiralis*, *Ascaridia galli*, Phytoconstituents.

Introduction

The characteristics property of medicinal plants is due to variety of complex chemical compounds and hence plants are referred as natural biochemical factories¹. Their therapeutic application can be established in light of modern medicine. In many countries, particularly those in tropical and subtropical regions, almost all the indigenous population is infected with hookworms or other helminthes. The gastro intestinal helminthes become resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases. Hence there is an increasing demand towards natural anthelmintics². In addition, worm infections are also a major cause for concern in veterinary medicine, affecting both domestic pets and farm animals³.

Wrightia tinctoria is a member of the family Apocynaceae, is a small to medium-size deciduous tree⁴. Traditionally *Wrightia tinctoria* commonly called as "Jaundice curative tree" in south India and plant possesses high medicinal value⁵. Crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. In Siddha system of medicine, it is used for psoriasis and other skin diseases⁶⁻⁹. The plant has been assigned to analgesic, anti-inflammatory and antipyretic activities and to be effective in the treatment of psoriasis¹⁰⁻¹¹. The literature survey revealed that no reports were found on the anthelmintic activity of the leaves extracts of *Wrightia tinctoria*. This prompted us to investigate the anthelmintic activity of *Wrightia tinctoria* leaves extracts.

Materials And Methods

Plant Material

Fresh leaves of *Wrightia tinctoria* was collected from TBGRI, Thiruvananthapuram during the month of March 2007. The plant was identified by Mrs. Amina Ali, Associate Professor and Head, Department of Pharmacognosy, Govt. Medical College, Calicut, Kerala, India. Voucher specimen (AA-34/10) is preserved in institute herbarium for future reference.

Preparation of Extract

Ethyl alcohol extract: The shade dried powdered leaves (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The extract was concentrated in vacuo to a syrupy consistency. The percentage yield of extract was found to be 2.9%.

Aqueous extract: The dried powders (24#) 100gm of the was taken in a 2000ml conical flask with 500ml of distilled water to which 10ml chloroform were added as a preservative. It was extracted up to 7 days with daily 2 hours stirring with the mechanical stirrer. After 7 days the extract was filtered through the muslin cloth and the marc was pressed and its filtrate dried in hot air oven at 45°C to a semisolid mass. It was stored in airtight container in a refrigerator below 10°C. The percentage yield of extract was found to be 3.1 %.

Animals:

The anthelmintic assay was carried out as per the method of *Ajaiyeoba et al*¹². Tape worm (*Raillietina spiralis*) and round worm (*Ascaridia galli*) were used as suitable models for screening of anthelmintic activity. Tape worm and round worms were obtained from intestine of freshly slaughtered fowls. These infested intestines of fowls were collected from the local slaughter house washed with normal saline solution to remove all faecal matter. Intestines were then dissected and worms were collected and kept in normal saline solution. 50 ml of formulation containing three different concentrations, each of alcoholic and aqueous extracts (10, 25 and 50 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both *Raillietina spiralis* and *Ascaridia galli*. Time for paralysis was noted when no movement of sort could be observed except when the worms were shaken vigorously. Time for death of worm was recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Test samples of the extract were prepared at concentrations 10, 25, 50 mg/ml in distilled water and six worms (same type) were placed in each petri dish. Piperazine citrate (10 mg/ml) was used as a reference standard and distilled water as control.

Result And Discussion

Preliminary phytochemical screening of ethyl alcohol extract revealed the presence of steroids, alkaloids, flavanoids, tannins and poly phenols. Aqueous extract contains alkaloids, flavanoids, tannins and poly phenols. The leaves extracts of *Wrightia tinctoria* displayed significant anthelmintic activity ($p < 0.05$) in dose dependent manner as giving shortest time of paralysis (P) and death (D) with 50 mg /ml concentration, for all two types of worms as shown in Table 1. Evaluation of anthelmintic activity was compared with reference standard piperazine citrate .The anthelmintic activity of piperazine citrate produced by increasing chloride conductance of worm muscle membrane produces hyperpolarization and reduced excitability that leads to muscle relaxation and flaccid paralysis.

Table No: 01. Anthelmintic activity of extracts of *Wrightia tinctoria* leaves.

Extract of Groups	Concentration mg/ml	Raillietina spiralis		Ascaridia galli	
		P	D	P	D
EA	10	28±1.52	58±1.05	24±1.29	55±1.05
	25	15±1.82	45±0.72	16±1.68	46±0.72
	50	10±0.60	29±1.02	10±0.60	31±1.02
AE	10	30±1.38	65±1.32	34±1.38	53±1.07
	25	24±0.76	46±0.72	22±0.76	40±0.72
	50	13±0.83	36±0.58	13±0.80	28±0.58
PC	10	10±1.04	34±0.68	10±1.04	34±0.68
Control	-	-	-	-	-

Each value represents mean ± SEM (n=6). Where EA: Ethyl Alcohol extract, AE: Aqueous extract, PC: Piperazine citrate, P: Time taken for Paralysis (min), D: Time taken for Death of worms (min).

Ethyl alcohol extract of *Wrightia tinctoria* shows the paralysis as well as death of worm in a less time as compared to piperazine citrate especially at higher concentration (50 mg / ml). While aqueous extract also shows significant activities. The ethyl alcohol and aqueous extract reveals the presence of flavanoids and polyphenolic compounds as one of the phytochemical constituents. Polyphenolic compounds show anthelmintic activity. Leaves indicated the presence of flavonoids, glycoflavones-iso-orientin and phenolic acids^{13,14}. Some of

the synthetic polyanthelmintics, eg: niclosamide, oxclosanide are shown to interfere with energy generation in helminth parasites by a coupling oxidative phosphorylation¹⁵. It is possible that phenolic compound in the extract of *Wrightia tinctoria* produce similar effect which needs to be investigated further. Another possible anthelmintic effect of tannins is, they can bind to free protein in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and cause death^{16, 17}.

Conclusion

The traditional use of leaves of *Wrightia tinctoria* as an anthelmintic have been confirmed as the leaves extracts displayed activity against the worms used in the study. This result lend the credence to the folkloric use of this plant in treatment against worms and show that *Wrightia tinctoria* could be exploited as a new potent anthelmintic.

Acknowledgement

The author's thankful to Mrs. Amina Ali, Associate Professor and Head, Department of Pharmacognosy, Govt. Medical College, Calicut, Kerala, India, for authentication of the plant specimen.

References

1. Thomas J., Development of Medicinal and Aromatic plants-Outlook for the next millennium., Indian J Arecanut Species Med Pl.,1(2),1999: 38-41.
2. Sathish B kosalge and Revindra A Fursule. Invetigation of anthelmintic activity of Thespesia lamps. Asian journal of Pharmaceutical and clinical Reserch.2(2),2009:69-71.
3. Alex Loukas and Peter J Hotez.Chemotherapy of helminth infections. In Goodmann and Gillmann's. The pharmacological basis of therapeutics,Edited by Laurance L Brunton.Mc Graw-Hill, New York.2006;11th ed:1073-74.
4. Kothari M J,Londhe A N ,Ethanobotany of Human Health Care of Chikhaldara,amaravathi district in Maharashtra State:In J K maheshwari Ed.: Ethanobotany and Medicinal Plants of Indian subcontinent,Scientific Publisher,Jodhpur,India,2000.
5. S.G. Joshi, Medicinal plants, Oxford and IBH publishing Co. Pvt. Ltd., New Delhi. 2000, 51- 52.
6. A. K. Nadkarni, Indian Materia Medica, Popular Prakashan, Bombay, 1976.
7. P.S. Varier, Indian Medicinal plants, Orient Longman Ltd., Madras, 1997.
8. M.J. Kothari, A.N.Londhe, Ethnobotany of Human Healthcare of Chikhaldara, Amravati District in Maharashtra State.: In J.K. Maheshwari Ed., Ethnobotany and medicinal plants of Indian subcontinent, Scientific Publishers, Jodhpur, India,2000.
9. M.J.Kothari, K.M. Rao , Ethnobotanical studies of Thane District Maharashtra, In, J.K. Maheshwari Ed.: Ethnobotany and Medicinal plants of Indian Subcontinent, Scientific Publisher,Jodhpur, India ,2000.
10. D.Ghosh, P.Thejmoorth, G.Veluchamy , Antiinflammatory, analgesic and antipyretic activities of 777 oil-a siddha medicine. Bull. Med. Ethanobot. Res 1985, 6, 141-154.
11. J.R.Krishnamurthi, S.Kalaimani, G.Veluchamy, Clinical study of Vetapalai (*Wrightia tinctoria*) oil in the treatment of Kalanjagapadai (Psoriasis). Journ. Res. Ayur. Siddha 1981, 2(1): 58-66.
12. Ajaiyeoba EO, Onocha PA and Olarenwaju OT. In vitro anthelmintic properties of *Buchholzia coriaceae* and *Gynandropsis gynandra* extract.Pharm Biol.39; 2001:217-220.
13. Daniel M, Sabnis SD, Chemotaxonomical studies on Apocynaceae. Indian J. Exp. Biol., 1978, 16(4): 512-513.
14. Daniel M, Sabnis SD, A chemotaxonomic appraisal of the slatus of Apocynaceae and Asclepiadaceae. Indian Bot. Repr., 1982, 1(2): 84-90.
15. Deore SL,Khadabadi SS, Kamdi KS, Ingle VP, Kawalkar NG,Sawarkar PS ,Patil UA and Vyas A J.In Vitro Anthelmintic Activity of *Cassia tora*. International Journal of ChemTech Research .2; 2009:177-179.
16. Mali RG and Wadekar RR. InVitro anthelmintc activity of *Baliopermum montanum Muell Arg* roots. Indian J Pharm Sci.2008:131-133.
17. Niranjana Sutar,Ranju Garai,Uma Shanker Sharma, Sharma U K and Amith Jaiswal.Anthelmintic activity of *Platyclusus orientali* leaves extracts.International Journal Of Parasitology Reserch.2010:01-03.