WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 4, Issue 2, 502-509. Research Article ISSN 2277– 7105

ANTHELMINTIC ACTIVITY OF THE LEAVES OF MURRAYA KOENIGII LINN AND MUSSAENDA FRONDOSA LINN.

Asha Kesari*[1] and Dr. Bhim Charan Maiti[2]

[1]Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Phase-II, G.B.Nagar, Chhend, Rourkela, Odisha: 769015

[2]Department of Pharmachemistry, Kanak Manjari Institute of Pharmaceutical Sciences, Phase-II, G.B.Nagar, Chhend, Rourkela, Odisha: 769015

Article Received on
10 Nov 2014,

Revised on 20 Dec 2014,
Accepted on 13 Jan 2015

***Correspondence for Author**
Asha Kesari
Department of Pharmaceutics, Kanak Manjari Institute of Pharmaceutical Sciences, Phase-II, G.B.Nagar, Chhend, Rourkela, Odisha: 769015

ABSTRACT

The aim of the present study was to evaluate the anthelmintic potential of crude extracts of leaves of Murraya koenigii and Mussaenda frondosa in different fractions namely petroleum ether, chloroform and methanol using Pheretima posthuma (Annelida) as test worms. Various concentrations (2.5-20.0 mg /ml) of the different fractions were tested in the bioassay which involved determinations of the time of paralysis (P) and the time of death (D) of the worms. Albendazole (10.0mg / ml) solution was included as standard reference and Tween 80 (0.1%) as control .The result of the present study indicated that the petroleum ether, chloroform and methanolic fractions of Murraya koenigii and Mussaenda frondosa significantly demonstrated the slowing down of the movement and caused death of the worms at a concentration of 20.0mg /ml as compared to the standard reference, Albendazole. MFC fractions showed the minimum time required for death. In conclusion, the traditional use of the Murraya koenigii and Mussaenda frondosa as an anthelmintic has been confirmed and further studies are suggested to isolate the active principles responsible for the activity.

KEYWORDS: Albendazole, Anthelmentic, Murraya koenigii, Mussaenda frondosa, Pheretima posthuma.

INTRODUCTION

Helminthic infections are now being recognized as cause of much chronic ill health and sluggishness among the tropical people .More than half of the population in the world suffers from worm infection of one or the other kind, which is a matter of great concern.

Traditional system of medicine reports the efficiency of several natural products eradicating helminthes. Keeping this in view, the present study deals with the evaluation of anthelmintic activity of the leaves of Murraya koenigii (Rutaccea) and Mussaenda frondosa (Rubiaceae).
Murraya koenigii (Rutaccea) is an unarmed, small aromatic tree with dark grey bark and closely crowded spreading dark green foliage .The roots, barks and leaves have been used since ayurvedic times for different therapeutic purposes.[1] The roots, barks and leaves are bitter, acrid, astringent, aromatic, demulcent, depurative, anthelmintic, febrifuge, stomachic, appetizing, carminative, anodyne, constipating, anti-inflammatory, antiseptic and tonic.[2].Ancient literature shows that it has been used in helminthes.[2]

Another garden plant Mussaenda frondosa (Rubiaceae) is a handsome erect or scandent shrub with grey barks ,leaves simple and the calyx lobes becomes enlarged into white ,pink or orange foliacecous structure , The plant is astringent ,sweet , expectorant, febrifuge, anti-inflammatory , vulnerary , alterant, demulcent, ophthalmic and cardiotonic.[3] The plant has shown several potential activities like the anti –microbial activity [4] ,antioxidant [5] , wound healing[6,7], hypolipidemic[8] and radical scavenging.[9] Siju et al (2010) has reported the anthelmintic activity in the ethyl alcohol and aqueous extract of the plant.[10]

MATERIALS AND METHODS

Plant collection and Authentication

The stems and leaves of Murraya koenigii and Mussaenda frondosa were collected from mature trees and its botanical identification was confirmed by Dr. M.K.Panigrahi, Professor and Dean , KMIPS and a voucher specimen was deposited in herbarium of KMIPS , Chhend colony , Rourkela ,Odisha.

Plant Extraction: The plant material of Murraya koenigii and Mussaenda frondosa were shade dried for several days and powdered material was extracted successively by petroleum ether (60^0- 80^0C) and with chloroform using soxhlet and by methanol by cold percolation method. It was evaporated and dried in vaccum to yield a dark coloured material soluble in

DMSO or Dimethyl Sulfoxide. All the fractions were preserved in the refrigerator till further use.

Worms Collection and Authentication: Indian earthworm Pheretima Posthuma (Annelida) was obtained as gift samples from Centre of P.G. Studies, Department of Microbiology, Orissa University of Agriculture and Technology, Bhubaneswar, Odisha.

Preparation of Test sample: Samples for in –vitro studies were prepared by dissolving 200 mg of each extract in 100 ml of distilled water using 0.1 % Tween 80 as suspending agent to obtain a stock solution .From this stock solution, different working dilutions were prepared to get concentration range of 2.5 , 5.0 ,10.0 and 20.0 mg /ml .

Anthelmintic Assay: The anthelmintic assay was carried as per the method of Ajaiyeoeba E.O.et al [11] with minor modifications. The assay was performed on adult Indian earthworm, Pheretima posthuma of length 8-10cms due to its anatomical and physiological resemblance with intestinal roundworm parasite of human being.[12-15] Due to easy availability, earthworms have been widely used for in vitro evaluation of anthelmintic compounds.[16 - 19]

50ml formulation containing concentrations, each of crude extracts of Murraya koenigii and Mussaenda frondosa in petroleum ether, chloroform and methanol were prepared in distilled water using 0.1% Tween 80 as suspending agent and six worms (same type) were placed in it. Time for paralysis was noted when no movement of any sort could be observed except where the worms were shaken vigorously .Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water (50^0C)[20 - 21] Albendazole (10mg/ml) was used as reference standard while 0.1% Tween 80 in distilled water as the control.

RESULT AND DISCUSSION

Preliminary phytochemical screening of crude extracts and its different fractions revealed the presence of flavanoids, alkaloids, saponins and tannins. As shown in Table 1 the methanolic extract of the two plants exhibited activity in dose-dependent manner giving shortest time of death within 20mg/ml. In most of the cases the initial movement was fast but slowed down with time, but paralysis was not seen and ultimately death was observed after some time. The reference drug, Albendazole showed the same after 48 minutes and immobilization in 29 minutes. This shows that the test drug had minimum effect on the muscles of the helminthes.

The benzimidazole exemplified by mebendazole and albendazole are versatile anthelmintic agents, particularly against gastrointestinal nematodes and hematodes where there action is not dictated by the systematic drug concentration .Appropriate doses of mebendazole and albendazole are highly effective against both larval adult stages of hematides. Immobilization and death of suspendible gastrointestinal parasites occur slowly and the clearance from the gastrointestinal tract may not be complete until a few days after treatment.

Benzimidazole produce many biochemical change in susceptible nematodes e.g. inhibition of mitochondrial fumarate reductase, reduced glucose transport and uncoupling of oxidative phosphorylation.[22] There is strong evidence that the primary action of these drugs is to inhibit mitochondrial polymerization by binding β –tubulin.[22]

The selective toxicity of these agents derives from the fact that specific high affinity binding to parasite β- tubulin occur at a much lower concentration than dose binding to the mammalian parasite. Thus the two identified mechanism of drug resistance to nematodes involve both a progressive loss of 'susceptible' β- tubulin gene isotypes together with emergence of a 'resistance' isotypes with a conserved point mutation that encodes a tyrosine instead of a phenylalanine at position 200 of β tubulin.[22]

The two extracts fractions demonstrated slowly down of the movement and caused death at a concentration of 20mg/ml in a time almost near to the reference drug, albendazole. Phytochemical analysis of the crude extract revealed the presence of tannins among the other chemical constituents contained within them. Tannins were shown to produce anthelmintic action [23] chemically tannins are polyphonolic compounds.[24] Some synthetic phenolic anthelmintic are reported to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation.[25] It is possible that tannins present in the extracts produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to the proteins in the gastrointestinal tract of the host animals [26] or glycoprotein on the cuticle of the parasite[27] and may cause death.

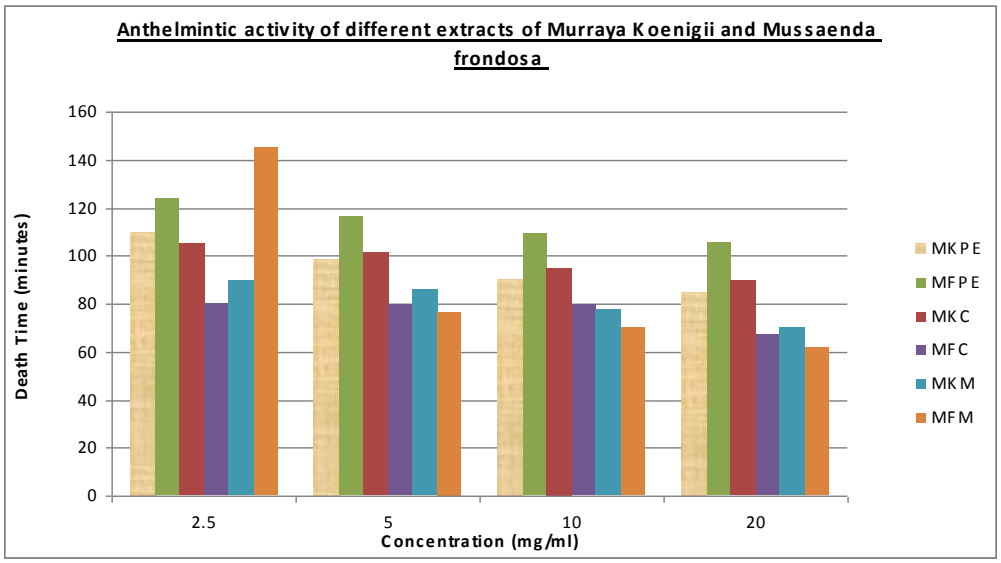
The traditional medicines hold a great promise as a source of easily available effective anthelmintic agents in people, particularly in developing countries including India. It is in the context that the people consume several plants or plant derived preparations to cure helminthic infections[28] e.g. decoctions of Murraya koenigii leaves are given to children to cure worms, without causing resistance to the worms .The origin of many effective drugs

have been found in the traditional medicine practices and in view of this it is important to undertake studies pertaining to screening of the folk medicinal plants for their claimed anthelmintic efficiency.

Table 1: Anthelmintic activity of different extracts of Murraya Koenigii and Mussaenda frondosa

Sl.no	Test Substance	Concentration Mg/ml	Time Taken	Time Taken for death (D)in minutes	
				Murraya Koenigii	Mussaenda frondosa
1	Control	-	-	-	-
2	Standard (Albendazole)	10.0	29+0.09	48±1.09	48±1.09
3	Petroleum Ether Extract	2.5 5.0 10.0 20.0	- - - -	109.50±0.43 98.33±0.42 90.00±0.37 85.00±0.26	123.92±0.48 116.33±0.33 109.33±0.33 105.50±0.43
4	Chloroform extract	2.5 5.0 10.0 20.0	- - - -	105.17±0.31 101.33±0.49 94.83±0.31 89.67±0.42	80.17±1.31 79.50±0.43 79.33±0.49 66.67±0.42
5	Methanolic Extract	2.5 5.0 10.0 20.0	- - - -	89.67±0.42 85.83±0.48 77.67±0.42 69.83±0.30	145.17±0.49 76.50±0.34 69.67±0.42 61.67±0.33

All Values represent Mean ± SEM .n = 6 in each group, $p < 0.01$ and $p < 0.05$



Graph 1: Anthelmintic activity of different extracts of Murraya Koenigii and Mussaenda frondosa

CONCLUSION

The traditional use of leaves of Murraya koenigii has been confirmed as the leaves, stem extract displayed profound anthelmintic activity in the study. Mussaenda frondosa leave extracts has shown good activity and can be promising newer medicine for use for the above action. Further it would be interesting to isolate the possible phytocontituents which may be responsible for the anthelmintic activity and to the possible the mechanism of action.

ACKNOWLEDGEMENT

The authors are grateful to Dr.P.K.Sahoo, Dean, School of Pharmaceutical Sciences, SOA University, Bhubaneswar, Odisha and Dr. T. K. Gaya, Reader, College of Teacher Education, Rourkela for completing this project.

REFERENCES

1. Kritikar KR ,.Basu BD , Indian Medicinal Plants, M/s Lalit Mohan Basu, Allahabad: 1935, pp. 472-474.
2. Varier's PS, Indian Medicinal Plants. M/s Orient Longman Limited, Madras: 1995, pp. 75.
3. Varier's PS, Indian Medicinal Plants, M/s Orient Longman Limited, Madras: 1995, pp. 86.
4. Jayasinghe ULB, Jayasooriya CP ,.Bandara BMR ,. Ekanayake SP , Merlini L., Assante G. , Antimicrobial activity of some Sri Lankan Rubiaceae and Meliceae Fitoterapia, 2002; 73 (5): 424-427.
5. Siju EN , Rajalakshmi GR . , Kavitha V.P. Joseph A. In Vitro Antioxidant Activity of Mussaenda Frondosa. Int.J. Pharm Tech Res., 2010; 2(2): 1238.
6. Patil Suhas A and Joshi VG. Evaluation of antibacterial and wound healing activity of leaves of Mussaenda frondosa linn. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2 (1):147-154.
7. Patil SA., Joshi V.G. and Sambrekar S.N .Evaluation of Wound Healing Activity of Isolated Compound Quercetin and Alcoholic Extract of Leaves of Mussaenda frondosa Linn. Research Journal of Pharmacognosy And Phytochemistry. 2011; 3(6): 266 – 271.
8. Wesley JJ, Jeyaananath Ji, Mohammed Ansar A, Ravikumar K. Hypolipidemic effect of Methanolic extract of Mussaenda frondosa linn. Leaves in high fat diet fed rats. Journal of Pharmacy Research. 2009; 2(4):579-581.

9. Koul S and Chaudhary A. Radical scavenging and antistress activity of Mussaenda frondosa roots (Rubiaceae). Pharmacologyonline. 2011; 1: 1091-1097.
10. Siju EN , Rajalakshm GR, Harira N, Sreejith KR, Sudhakaran S, Munee EK and Premalatha K. In-Vitro Anthelmintic Activity of Mussaenda frondosa Research J. Pharm. and Tech, 2010; 3 (1):151-153.
11. Ajaiyeoba EO, Onocha PA and Olarenwa OT, In vitro anthelmentic properties of Buchholzia coriaceae and Gynandropsis gynandra extract. Pharm.Biol, 2001; 39(3): 217- 220
12. Vidyarthi RD, A textbook of Zoology, S.Chand and Co. New Delhi, 1967 14th edition: pp- 329-70.
13. Thorn GW, Adams RD, .Brawnwald E, Isselbacher KJ and Petersdorf RG Harrison's Principles of Internal Medicine Mcgraw Hill Co., New York: 1977, pp.1089
14. Vigar Z, Atlas of Medical Parasitology,P.G.Publishing House , Singapore, 1984 pp.216
15. Chatterjee KD, Parasitology, Protozoology and Helminthology , Guha Ray S Sree Saraswaty Press Ltd, Calcutta, 1967, 6th edition: pp.168-169
16. Sollmann T. Anthelmintics: Their efficiency as tested on earthworms. J Pharmacol. Exp. Ther .1918; 12: 129-170.
17. Dash GK, Suresh P, Sahu SK, Kar DM, Ganapaty S and Panda SB. Evaluation of Evolvulus alsinoides Linn. for anthelmintic and antimicrobial activities. J. Nat. Rem. 2002; 2(2): 182 - 85.
18. Szewezuk VD, Mongelli ER and Pomillo AB.Antiparasitic activity of Melia azadlrach growing in Argentina. Mole. Med.Cliem .2003; 1:54-57.
19. Shivakar YM, .Kumar VL, Anthelmintic activity of latex of Calotropis procera. Pharm boil. 2003; 41(4):263-65.
20. Mali RG, Hundiwale JC ,Sonawane RS , Patil RN and Hatapakki. BC Evaluation of Cappon's Decidua for anthelmentic and anti microbial activities .Ind.J.Nat.Prod 2004; 20(4):10-13.
21. Mali RG, Shailaja Mahajan and Patil KS .Anthelmintic activity of root bark of Capparis Spinosa . Ind.J.Nat.Prod. 2005; 21(4):50-51.
22. Tracy JW, Webster,Jr LT, Drugs used in the Chemotherapy of Helminthiasis In: J. G. Hardman, L.E. Limbird, eds, A.G.Gilman, Goodman and Gilman's The Pharmocological basis of Therapeutics, 10th ed. The Mc.Graw –Hill Companies: pp.1126.

23. Niezen JH, Waghorn GC, Charteston WAC.Growth and gastrointestinal nematodes parasitism in lambs grazing either Lu cerne (Medicago sativa) or sulla (Hedysarum coronarium) which contain condensed tannins .J.Agri .Sci 1995; 125:281-89.
24. Bate – Smith EC .The phenolic constituents of plants and their taxonomic significance, dicottyledons .J.Linn . Soc. Bot, 1962; 58: 95-103.
25. Martin RJ .Mode of action of anthelmentic drugs, 1997; 154:11-34.
26. Anthnasiaduo S, Kyriazakis F, .Jackson RL and Coop. Direct anthelmentic effects of condensed tannins towards different gastrointestinal nematodes of sheep, in-vitro and in-vitro studies .Vet .parasital. 2001; 99: 205-19
27. Thompson DP and Geary TG .The structure and function of helminth surface .In:J.J.Marr, eds, Biochemistry and Molecular Biology of Parasites 1st ed. New York, Academic Press:1995, pp. 203-232.
28. Satyavati GV, Use of Plant Drugs in Indian Traditional System of Medicines and their relevance to Primary Health Care .In by : N.R.Farnsworth , H.Wagner, eds .Economics and Medical Plant Research ,Vol.IV ,London , Academic Press Ltd: 1990, pp.190-210.