J. Chem Soc. Nigeria, Vol. 43, No. 4, pp 904 -914 [2018]

ANTHELMINTIC PRINCIPLES FROM THE TUBEROUS ROOTS OF *Neorautanenia mitis* (A. Rich) Verdcourt – Papilonaceae

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Received 22 July 2018; accepted 24 September 2018, published online 06 November 2018

Abstract
In Nigeria, *Neorautanenia mitis* is used for treating intestinal parasites caused by tapeworm. Pulverised tuber of *N. mitis* was exhaustively and successively extracted using the *n*-hexane, chloroform and MeOH. Extracts and isolated compounds were evaluated for anthelmintic activity against dwarf tapeworms (*Hymenolysis nana*) and the larva of hookworm using the *in vivo* and *in vitro* models. *In-vivo* anthelmintic activity of the crude n-hexane, $CHCl_3$ and MeOH extracts of *N. mitis* exhibited 100 % paralysis of the worms at 100 mg/kg. LD_{50} of the cude extracts and *Albendazole* (anthelmintic drug) were 188. 5 and 45.25 µg/mL respectively. Isolated compounds from *N. mitis*: Neoraudiol (**1**), neoduline (**2**), neotenone (**3**), rautandiol (**4**), pachyrrhizine (**5**) and 12a-hydroxy neotenone (**6**) displayed concentration-dependent anthelmintic activities against the tapeworm (*Taenia solium*) at 25, 50, 80 and 100 mg/mL. Structure-activity relationship was established. The results of the study provide scientific justification for the use of *N. mitis* in combating anthelminthes in Nigeria.

Key words: *Neorautanenia mitis*, isolated compounds, Papilonaceae, Anthelminthic activity, Parasitic worms, anthelminthes.
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Introduction

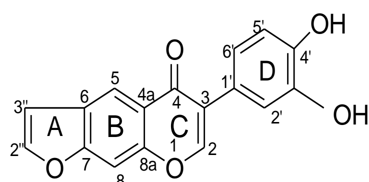
In developing countries, medicinal plants have been an integral part of the people. Plants with anthelmintic principles are reported in herbal pharmacopeias, directories and scientific journals [1, 3, 4, 10, 17]. Nigeria has abundant flora and fauna which are yet to be explored for drugs [9]. Traditional medicines from plants are a cheap and main source of providing drugs to citizens against diseases endemic to the tropical countries. Parasitic diseases, particularly helminth infections are among

the public health problems of tropical countries, infecting man, domestic animals and wild life. In 1995, death from helminthiases was about 200,000, hence their prevalence constitutes major health concern [22]. Helminthiases constitute serious causes of mortality and morbidity especially among peasants and rural dwellers in Nigeria, due to poor sanitation, malnutrition, social-economic status and poor living standards [22]. Helminthiases predispose body to fungal, bacterial and HIV diseases [5, 15]. A world estimate reports that one-third of the global population harbor ascaris [22]. Helminthiases causes blood sucking, damage to gastro-intestinal mucosa by nodule formulation, depletion of nutrients of hosts and chronic irritation.

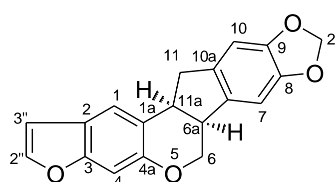
Isoflavonoids, polyphenols, terpenes, glycosides, flavonoids, imidazole thiazoles, etc are interesting phytoalexin adjudged and reported as anthelminthic candidates[10, 18]. Various morphological parts of medicinal plants have been reportedly used in different trado-medical care as anthelmintic remedies [3, 8].

Neorautanenia, is one of the largest genera of the Papilonaceae (Fabaceae) with less than ten species [9] and are widely reported for antimicrobial, anti-inflammatory, cytotoxicity, anthelmintic activities [6, 12, 13, 19 and 21]. *N. mitis* is a very rich source of bio-flavonoids, pterocarpans and coumarin [6, 13, 19 and 21]. Flavonoids are attracting more attention as phytoalexin due to their structural uniqueness and interesting biological activities [19]. The morphology of *N. mitis* has been documented [9]. In the past, chemical constituents from the tuberous roots of *N. mitis* afforded flavonoids Neoduline, Neotenone, Neoraudiol, Rautadiol, Pachrrhizine and 12a-hydroxylneotenone [2].

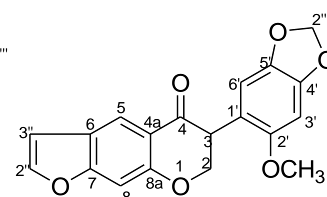
Within the scope of our quest for anthelmintic agents from arid and tropical rain forests in Nigeria, we investigated the extracts and compounds isolated from the tuberous roots of *N. mitis*. Herein, we report the anthelmintic activities of the n-hexane, $CHCl_3$ and MeOH extracts and their active isolates from the root tubers of *N. mitis*. To the best of our knowledge, this is the first scientific documentation on the anthelmintic agents from *N. mitis*, an indigenous plant used in the trado-medical practice in Nigeria for treating gastro-intestinal disorder by the Idu natives of Abuja, Nigeria (Personal communication).



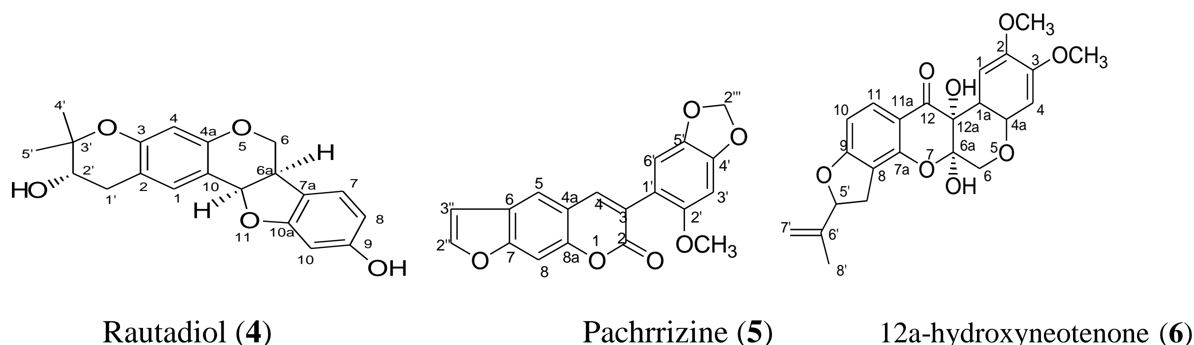
Neoraudiol (**1**)



Neoduline (**2**)



Neotenone (**3**)



Rautadiol (**4**) Pachrrizine (**5**) 12a-hydroxyneotenone (**6**)

MATERIALS AND METHODS

General Experiment

Melting points (mp) are reported uncorrected. Adsorption Chromatography were conducted on silica gel adsorbent. Spectroscopic data (IR, Uv-Vis and NMR) were taken on standard spectroscopic equipment as previously reported [2].

Chemicals used were obtained from Sigma Aldrick (England). The chemicals used were of IP/BP specification.

Plant collection, authentication and preparation

The tuberous roots of *N. mitis* were collected from the rocky soil along Suleja-Abuja road, Niger State, Nigeria in June, 2007 with the assistance of Mr. O. A. Ohaeri of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu Abuja, Nigeria. Voucher specimen was deposited in the herbarium of NIPRD with accession number NIPRD 11225.

The root tuber of *N. mitis* were cut into small pieces, washed thoroughly with distilled water, dried partially for 1 h under sunlight, to prevent rottening of sample and finally shade dried at ambient temperature in Chemistry Research laboratory for 2 weeks. Dried tubers were pulverized with mechanical grinder in wood extraction laboratory at the University of Ibadan, Ibadan. The ground plant material was stored in air-tight sack, free from moisture.

Extraction of pulverized plant material

2 kg of pulverized tubers of *N. mitis* were loaded into the aspiration bottle, fitted with extraction gadgets. The sample was exhaustively extracted with n-hexane, $CHCl_3$ and MeOH in succession by percolation. Each extract was concentrated in vacuo by means of rotary evaporator to yield 28, 35 and 135 g of the extracts respectively.

Biological Evaluation

Preparation of drugs and Chemicals

Chemicals used for the bio-assay were analytical grade, procured from Aldrich Chemical (England). 0.9 % of normal saline was prepared. Extracts and standard drugs used were dissolved in 1.0 % of dimethyl sulphoxide (DMSO) in normal saline (vol/vol). Albendazole purchased from Dimax Pharmaceutical (Ibadan, Nigeria) was prepared at three different concentrations of 25 mg/mL, 50 mg/mL and

100 mg/mL in distilled water and served as the reference drugs for the anthelminthic assay in this study.

Acute toxicity and determination of LD_{50} of extracts of *N. mitis*

The LD_{50} of the crude extracts were evaluated as described with slight modifications [7]. Extracts of *N. mitis* were applied orally at doses of 625, 2,500 and 5000 mg/kg p.o. to six experimental animals in each group. Toxicity such as death, physical appearance and or behavioral animal changes was observed for 96 h after administration of extracts. The LD_{50} was determined by geometric means of dose that led to 100% lethality and the maximum dose with zero toxicity.

In vitro parasitic culturing of hookworm eggs

Larvae from infested hookworm eggs of infested stools were cultured in line with the procedure of Harada and Mori [20]. 0.7 g of fecal matter was applied on a filter paper, inserted into a test tube containing distilled water. Samples were incubated at 25°C for 8 days. After developing the larval to a stage for the assay, they were centrifuged at 400 rpm for 20 min and concentrated. The larval count was conducted and the number of larval was adjusted to a concentration level of one larva per microliter.

In *vivo* anthelminthic activity of extracts from *N. mitis*

In-vivo anthelmintic assay was assayed in line with the procedure of Gill *et al*. [11], using a 96-well microliter plate on the third stage larvae of hookworm. Stock solution of crude extract of *N. mitis* and *albendozole* (anthelminthic drug) were prepared at 100 an 200 mg/mL in 1 % DMSO and were serially diluted by two-fold to produce different dilution concentrations. The aliquots were added at a dilution of 1% molten agar in 200 µL in the individual walls of a 96-well µL plate, this gives concentrations in two fold dilution as: 1, 0.5, 0.25, 0.125 and 0.0625 mg/mL for the extracts and 0.2, 0.1, 0.05. 0.025, 0.0125 mg/mL for *albendozole*. 30 larvae in 30 µL of distilled water were added to each well, and the plate was incubated in the dark at 25°C for 48 h of incubation. Larvae were tested for motion by adding 40 µL of warm water at 50°C to each well, control larvae and those showing no effect with the extracts and the standard drug indicate deparatization. The standard drug and the larvae exhibited a raid sinusoidal motion, while the extracts and compounds assayed either remained motionless or a typical twisting motion. Larval with sinusoidal motions were counted. Anthelminthic assay were carried out in triplicate.

In *vitro* anthelminthic activity of extracts from *N. mitis*

In vitro anthelminthic assay was conducted using experimentally infected albino mice maintained in the animal house. Eggs of experimentally adult dwarf tapeworms, (*Hymenolysis nana*) were obtained from the intestine of infected mice by dissection; dose of infection was adjusted to 800 eggs in 0.1

mL of normal saline. Each experimented mouse was infected orally by stomach tube. 2 weeks post-infection fresh fecal samples from each infected mice were collected and examined for shedding of ova. Mice not shedding ova of *H. nana* were discarded from the reports. Infected mice were randomly allocated to 5 control and experimental (Groups I-V). For each extract and isolate group I (positive control) with 5 mice, were treated with standard drug at 250 mg/kg body weight. Group II, also with 5 mice served as negative control and received normal saline via the same route. Groups III-V each with 5 mice was assayed for anthelmintic potency of the extracts and on compounds 1-6. During administration, all the mice were sacrificed to evaluate % deparasitization, using the expression:

% deparasitization= N-n/Nx100;

N= number of worm count in the negative control

N= number of worm count in the positive control

In *vitro* anthelminthic activity of isolated compounds from *N. mitis*

Anthelmintic potentials of the isolated compounds were directly evaluated using human tapeworm (*Taenia solium*), gotten from abattoir, at the slaughter slab, *Odo-eran*, Abeokuta, Ogun State, Nigeria. The anthelmintic procedure utilised was as described by Azuzu *et al*., [10].

Statistical analysis

Data are presented as the mean ± SEM from three separated experiments. Statistical analyses were performed using the Bonferroni t-test method, after ANOVA for multigroup comparison and the student's t-test method for group comparison. $P < 0.005$ was considered significant. Analysis of linear regression (at least 3 data within 20-80 % inhibition) was used to calculate LD_{50} values.

RESULTS AND DISCUSSION

Medicinal plants represent a rich source of anthelminthic agents (Lasisi and Idowu, 2014). Medicinal plants are used as medicines in different countries are a source of many potent and powerful anthelmintic drugs (Azuzu, 1999 and Lasisi *et al*., 2012). Extracts and isolated compounds (**1-6**) from the tuberous roots of *N. mitis* were assayed for anthelminthic activity in this study.

The acute toxicity and LD_{50} of the three extract revealed no acute signal of toxicity or mortality among experimental animals at varying dose levels (Table 1). It can be inferred directly from this result that since no record of death was recorded at the maximum lethal, the LD_{50} was higher than 5000 mg/kg body weight.

Table 1 **LD_{50} of the extracts of *N. mitis* and *Albendazole* drug using global sigmoidal model curve**

Extracts	Log LC_{50}		LC_{50}(µg/mL)		
	Best fit	Standard Error	Best fit	95 % CL	R^2
N-hexane	2.243	0.0325	23.150	189-250	0.9301
$CHCl_3$	2.357	0.0330	22.890	191-249	0.9305
MeOH	2.382	0.0328	22.90	191-252	0.9410
Albendazole	1.900	0.0345	51.255	43.75-62.50	0.9540

I*n vitro* larval mortality assay was carried out on the *n*-hexane, $CHCl_3$ and MeOH extracts of *N. mitis* assayed against the infective stage of the hookworm in this study. The best-fit LC_{50} values for the extracts, isolated compounds and standard drug (Albendozole) exhibited 225. 7 and 51.33 mg/mL (Table 2).

Table 2 **Worm count, % deparasitization and host clearance after treated with crude extracts of *N. mitis***

Animal Groups	Administered dosage	Mean number of Parasites	% deparasitization	% Host Cleared
I	70 mg/kg	$1.3 \pm 0.50^{*a}$	40	40
II	1000 mg/kg	$0.00 \pm 0.00^{*b}$	100	100
III	2000 mg/kg	$0.00 \pm 0.00^{*b}$	100	100
IV	25 mg/kg	$0.00 \pm 0.00^{*b}$	100	100
V	Vehicle	2.00 ± 0.32	0	0

PQ = Praziquantel

Mean number of parasites with significant difference at $P < 0.05$, a, b represent significant difference at $P < 0.01$ for LSD multiple comparison test.

In vivo anthelminthic assay on the extracts revealed that the crude extracts of *N. mitis* significantly reduced the worm count at $P < 0.05$, compare with the negative control (Table 3). The % deparasitization and the % parasite clearance of mice treated with 100 mg/kg of the extracts were at 100 %. The mice in the negative control group had mean parasites count of worms at necropsy.

Table 3 Worm count, % deparasitization and host clearance after treatment with isolated compounds from *N. mitis*

Isolated Compounds	Mean number of Parasites	% deparasitization	% Host cleared
Neoraudiol (**1**)	0.00 ± 0.25*[a]	100	100
Neoduline (**2**)	0.00 ± 0.00*[b]	100	100
Neotenone (**3**)	0.00 ± 0.00*[b]	100	100
Rautadiol (**4**)	0.00 ± 0.00*[b]	100	100
Pachrrizine (**5**)	0.00 ± 0.00	100	100
12a-hydroxyneotenone (**6**)	0.00 ± 0.00*[a]	100	100
Albendazole (Vehicle)	2.00 ± 0.32	0	0

The anthelmintic survey of compounds **1-6** conducted at 25, 50, 80 and 100 mg/mL exhibited varying degree of paralysis. (Table 4). Even at minimum concentration of 25 mg/kg, neoraudiol (**1**) paralyzed the worm assayed at a very short time. The degree of paralysis was found to be concentration dependent in each case assayed (Table 4). Neoraudiol (**1**) exhibited high morbidity at lower concentration compare with other isolated compounds. Meanwhile, all isolated compounds displayed higher anthelmintic potency compared with crude extracts. Perhaps at the isolated level of their respective extracts, higher concentration of the active constituents from the extracts potentiates higher activity. Meanwhile, anthelminthic drugs act by causing paralysis of the worms, which is eventually ejected from the body system by a purge [16].

Table 4 Anthelminthic Activities of isolated compounds of *N. mitis* on *Taenia solium* at different concentration

Conc mg/mL	**COMPOUNDS**	**1**		**2**		**3**		**4**		**5**		**6**	
		P	**D**	**P**	**D**	**P**	**D**	**P**	**D**	**P**	**D**	**P**	**D**
25		24.3 ± 0.27	28.6 ± 0.26	29.9 ± 0.35	31.3 ± 0.42	30.2 ± 0.21	34.3 ± 0.33	34.3 ± 0.24	38.2 ± 0.27	29.3 ± 0.27	32.6 ± 0.22	27.4 ± 0.11	29.4 ± 0.33
50		22.3 ± 0.32	25.3 ± 0.44	27.4 ± 0.28	29.4 ± 0.44	28.3 ± 0.27	32.4 ± 0.23	33.3 ± 0.19	36.3 ± 0.26	27.4 ± 0.15	29.1 ± 0.53	26.2 ± 0.13	28.3 ± 0.42
80		20.5 ± 0.41	22.3 ± 0.50	25.2 ± 0.27	27.9 ± 0.33	27.4 ± 0.22	29.4 ± 0.22	30.1 ± 0.44	32.3 ± 0.44	25.4 ± 0.25	26.3 ± 0.37	24.5 ± 0.22	26.3 ± 0.33
100		18.4 ± 0.35	20.7 ± 0.32	23.3 ± 0.40	25.3 ± 0.24	26.6 ± 0.29	28.1 ± 0.20	29.3 ± 0.40	31.7 ± 0.22	24.3 ± 0.22	28.2 ± 0.34	22.3 ± 0.26	25.2 ± 0.11
Conc (mg/mL)	***Albendazoles***	**P**	**D**										
25		20.6 ± 0.28	22.3 ± 0.23										
50		17.2 ± 0.22	25.4 ± 0.44										
80		14.7 ± 0.44	20.3 ± 0.25										
100		10.2 ± 0.22	18.7 ± 0.55										

P = paralysis time in mins; **D** = Time of death in mins

In this study, the tuberous root and compounds isolated from *N. mitis* were found to cause paralysis of the worms. An implication of this is to the effect that

purgative may not be necessary to expel the worms. Several natural products derived from medicinal plants have been reported for the control of helminthiasis. Alkaloids (Echitamine) isolated from *Alstonia bornei* and flavonoids gotten from Citrus acida, Mustard oil heteroside are potential anthelmintic [18]. The unique structure of flavonoids and coumarins, with the reactive carbonyl adjoining aromatic moiety constitutes major activities reported in flavonoids as anthelmintic and antimicrobial candidates. These functional groups interracts with host parasites, and denatured them in a form that rendered them inactive [18]. The present study which evaluated the extracts and active principles from *N. mitis* gave scientific justification for the folkloric usage of *N. mitis* in ravaging helminthiasis by Idu natives of Abuja, Nigeira. Hence, the flavonoids and polyphenols assayed in this study are good candidate for a drug lead delivery in helminthiasis.

Conclusion

Anthelminthic assay conducted on extracts and active compounds from *N. mitis* are an indicative of anthelmintic potential of the plant. Hence, a scientific justification is provided in this study for the traditional use of the extracts of *N. mitis* for treating worm infections.

Acknowledgement

We are grateful to Mr Ohaeri of the Nigerian Institute of Pharmaceutical Research and development, Abuja, Nigeria for the collection of plant material used in this study. Particularly, we extend our gratitude to National Institute of Horticulture and Research (NIHORT) for using their facilities for this study. The management of the University College Hospital (UCH), Ibadan (Parasitology Unit) is extensively thanked for their contributions in this study.

REFERENCES

1. A. A. Lasisi, D.A.,Ojo, M. O. Olayiwola, and S. A. Adebisi (2011). In-vitro anthelmintic and antibacterial properties of the leaf hexane isolates of *Pyrenacantha staudtii* EngL (Icacinaceae). ***Journal of Herbal Practice and Technology***, 1, 6-12.

2. A. A. Lasisi and A. Adesomoju (2015). Neoraudiol, a new isoflavonoid and other antimicrobial constituents from the tuberous roots of *Neorautanenia mitis* (A. Rich) 3. Verdcourt, ***Journal of Saudi Chemical Society***. 19 (1), 404-409.

3. A. A. Lasisi and Oluwafunmilayo, Idowu (2014). In vitro anthelmintic and cytotoxic activities from the stem barks of *Berlinia confusa* (C. Hoyle) and identification of its active constituents, ***Journal of Saudi Chemical Society*****,** 18 (6), 939-944

4. A. A**.Lasisi**, M. A. Olayiwola, and E. O. Dare (2012). Evaluation of anthelmintic activity of the stem bark extract of *Bridelia ferruginea* (Benth)-Euphorbiaceae, ***International Journal of Chemical Sciences*** **(IJCS)**, 5 (1), 120-122.

5. B. D. Cabrera (1981). Ascaris: Most popular worms, World Health Organisation Report, March, pp 8.

6. C. C. Joseph, M. M. Ndolie, R. C. and M. H. Malima Nkunya (2004). Larvacidal and mosquitocidal extracts, a coumarin

and pterocarpan from N. mitis. **Transaction *of the Royal Society of Tropical Medicine and Hygiene***, 98, 451-455.

7. D. A. Lorke (1983). A new approach to practical acute toxicity testing, ***Arch. Toxicol***, 154, 275-287.

8. H. J. Hoskeri and V. Krishna (2011). Anthelminthic and Bactericidal Activity of Extracts from *Flaveria trinervia* (Spring C. Mohr; ***European Journal of Medicinal plants***, 1 (4), 153- 61.

9. H. M. Burkhill, (1995). The useful plants of West tropical Africa, J-L, vol.3. Royal Botanic Garden, Kew, Great Britain, 1400-1410.

10. I. U. Azuzu; A. I Gray and P. G. Waterman (1999). The anthelmintic activity of D-3-*0*- methyl hiroinositol isolated from *Poliostigma thonningii* stem bark , ***Fitoterapia***, 70, 77- 79.

11. J. H.Gill, J. M. Redwin, J. A. Van and E. Lacey (1991). Detection of resistance to ivermectin in *Haemonchus contortus*, ***Intl J. Parasitol***., 21, 771-776.

12. J. Leticia, A. Mariam and M. Manuel (2008). Pterocarpans: Interesting natural products with antifungal activity and other biological properties. ***Phytochem Rev.***, 7, 125-154.

13. L. Cromble, and D. A. Whiting (1962). The constitution of neotenone and dolichone: biogenetic connection in the sub-family Papilonaceae. **Tetrahed. Letters**, 18, 801-804

14. O. R.Correc and Bethonon, J. (2006). Contrasting in urban and rural environments in ***Brazil, Intl J. Parasitol***., 36, 1143-1151.

15. P. A. J Jansen (1974). Recent advances in the treatment of parasitic infections in man. ***Prog Drug Res.***, 18, 198-205

16. P. Lechet, F. Bisseliches, F. Bourerias and H. Denchy (1978). *Pharmacologie Medicale,* 3rd Edition, Masson, Paris, p 201-204.

17. S. Brooker, , N. Alexander, S. Gaper, R. A. Moyeed, J. Stander, F. Fleering, P. J. Hofez,

18 S. Nitinkumar, M. Shetty; J., Imtiyyaz, Ahmed and A. Chuljin (2010). Synthesis, Anthelminthic and Anti-inflammatory Activities of some novel Imidazoles sulfides and sulphones. ***Bull. Korean Chem. Soc***., 31 (8), 2337-2340.

19. S. Yojiro, S. Nobuko, T. Masahiko, N. Yuka, F. B. Kenneth, W. Xihong, M. C. Gordon and Kuo Hsiung, (2006). Rautandiols A and B, pterocarpans and cytotoxic constituents from *Neorautanenia mitis*. ***J. Nat. Prod.***, 69, 397-399.

20. U. Harada and O. Mori (1955). A new method for culturing Hookworm, ***Yonago Acta Med***, 1, 177-179.

21. V. P. Luc, D. K., Nobert, M. Jean-Pierre, G. Athanse and S. Niceas (1987). Isolation and structural elucidation of potentially insecticidal and acaricidal isoflavone-type compounds from *Neorautanenia mitis*. ***J. Nat. Prod.***, 50 (3), 349-356.

22. WHO Publication (Sourced from S. K. Sing and S. Sharma (1995). Current status of Helminth Chemotherapy, Wiley eastern Limited Publisher, p 110-7