European Journal of Medicinal Plants
13(1): 1-9, 2016, Article no.EJMP.17675
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation and Phytochemical Analysis of *Prosopis africana* and *Erythrina senegalensis* Used against Immature Stages of *Schistosoma haematobium*

P. G. Rwang[1*], J. P. Fabiyi[2], M. Suleiman[2] and K. P. Mercy[3]

[1]Federal University Lafia, Nigeria.
[2]Abubakar Tafawa Balewa University, Bauchi, Nigeria.
[3]University of Jos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/17675
Editor(s):
(1) Daniela Rigano, Department of Chemistry of Natural Compounds, University Federico II of Naples, Italy.
(2) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) M. Angeles Calvo Torras, Universitat Autonoma De Barcelona, Spain.
(2) Alli Smith Y.R, Ekiti State University, Nigeria.
(3) Maha Mohamed Eissa, Alexandria University, Egypt.
(4) Anonymous, Mexico.
Complete Peer review History: http://sciencedomain.org/review-history/13583

Original Research Article

Received 23rd March 2015
Accepted 11th November 2015
Published 7th March 2016

ABSTRACT

Seventeen groups of laboratory mice were infected with 200 cercariae of the helminth parasite, *Schistosoma haematobium*, per mouse, using the paddling technique. Mice in sixteen groups were subsequently treated with the plant extract of *P. africana* and *E. senegalesis* at weekly intervals while the seventeenth group was kept as infected control. A single oral dose of 40 mg/kg body weight was administered. The efficacy of the extracts against different ages of development of the disease was measure by mean percentage worm reduction; mean percentage reduction in tissue egg count and evaluation of hepatosplenic disease. The helminth parasite was highly susceptible to the extracts administered on days 0, 7, 14, 42 and 49 and showed cure with normal livers and spleens; however, mice treated on day 21, 28 and 35 with the extracts gave ineffective results'. Cure rate, given by percentage worm reduction, ranged from 96% - 98% and 94% - 96% for both *Prosopis africana* and *Erythrina senegalensis* respectively. The two plants showed anti-helminthic activity against urinary Schistosomiasis and phytochemical screening of the extracts revealed the

**Corresponding author: E-mail: pamrwang@gmail.com;*

presence of Tannins, Saponins, Anthraquinones, Cardiac-glycosides, carbohydrates, and steroids, while terpenes were absent.

Keywords: Evaluation; phytochemical; P. africana; E. senegalensis; Schistosomiasis.

1. INTRODUCTION

Schistosomiasis, also known as bilharziasis, is one of the most prevalent tropical parasitic disease in the world that leads to chronic ill health. Schistosomiasis has been recognized since the time of the Egyptian Pharaohs. Theodor Bilharz, a young German pathologist from whom the disease took its original name, Bilharziasis, eventually discovered the worms responsible for the disease in 1851. These parasitic infections constitute a major public health problem in the rural areas and continue to rank high in other developing countries [1]. Parasites, according to [2] cause misery, affect general health, causing growth retardation, social-economic problems, declining productivity and malnutrition. Some may even cause death when ectopic sites are invaded or when vital malfunction result.

Several species in the genus *Schistosoma* infects humans causing schistosomiasis, they include: *Schistosoma mansoni, S. haematobium, S. japonicum, S. mekongi, S. intercalatum.*

They live in the blood vessels of man and other animals. The adults do not multiply in the body but can remain living upto 30 years and, the female releases eggs that produce tissue damage. Some eggs leaves the body in the urine or faeces and hatch in water to liberate the miracidium larva, which infects freshwater snail, multiply asexually to produce free swimming cercariae larvae [3].

1.1 Mode of Infection and Life Cycle

Man become infected by wading in Cercariae infested waters. The penetrate the skin, shedding their tails in the process and reach a blood vessel where they enter the circulatory system [4].

The early stage symptom of schistosomiasis includes: skin rash and bronchial cough. Later, afternoon fever, anaemia, and it is also indicated either by the presence of blood in the urine in the case of urinary schistosomiasis or stool in the case of intestinal infections, by initially a typical symptoms which can lead to serious complications involving the liver and spleen [2]. People are infected by exposure to water containing infective larvae during used in normal daily activities such as personal hygiene and swimming, or by professional activities such as fishing, rice cultivation and irrigation water containing the infective larvae (cercariae). Many infections are symptomatic.

These complications are often on the vital tissues and organs of the body and are due to the presence of eggs of the adult parasite which induces the disease. Hence, chemotheraphy remains one of the main approaches to control the disease [5]. At the moment Praziquantel (PZQ) is the drug of choice for the treatment of schistosomiasis and many intestinal worms and it is considered a safe drug with only mild side effects [6]. However, [7] found severe liver lesion attributable to dead worms after praziquantel treatment of goats infected with *Schistosoma bovis*.

Apart from the minor side effects caused by praziquantel, another problem is the development of resistance to the drug. For example, resistance to drug has been observed in the laboratory [8]. In addition [9] noted resistance in the field in Egypt. Concerned about this negative development, it has become imperative to seek for new drugs that may be effective, safe and environmentally friendly. Praziquantel is an inorganic drug whose continuous excretion into the environment may be harmful. With current global concern about the safety of the environment, it would be advantageous to have drugs developed from organic sources, especially those of plant origin. Most plants possess therapeutic and pharmaceutical properties, which are due to the type of the chemical substances they synthesize and store during their normal metabolic activities. These include compounds such as Tannins, Saponins, Anthroquinones, Cardiac glycosides, Carbohydrates and Steroides that are utilized as food by man and other animals and also other compounds that exert physiological effect on them.

There have been a number of claims [10] regarding the efficacy of some local indigenous plants in the treatment of schistosomiasis by traditional healers. Among such plants are the

Prosopis africana and *Erythrina senegalensis.* As these will therefore be assayed and their efficacy established; it is hoped that this will provide a basis for the development of an effective, safe and environmental friendly treatment of schistosomiasis.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Plant materials were collected during the dry and hot condition/season of the study site-Bayara, in Bauchi L.G.A of Bauchi State, Nigeria with the permission of the forestry Department of the State. Plants collected were *Prosopis africana and Erythrina senegalensis* by the helped of a Taxonomist. Collections were made in the day time.

These plants were chosen based on their medicinal properties following literature searches which have been previously identified by a Taxonomist [11].

2.2 Preparation of Plant Material for Extraction

Only the mature leaves of the plants were collected (because mature leaves contain more concentrated compounds with active ingredients) and shade dried. The dried leaves were pounded to powder in a wooden mortar and stored in labeled containers.

2.3 Extraction Crude Plant Extract

Powered plant material was mixed with distilled water and methanol solution in separate containers of one litre pyrex conical flask each at a ratio of 1:5 weight per volume (w/v) according to the procedure described by [12]. Both mixtures were separately allowed to stand for 48 hours at ambient temperature and the suspension was filtered through layers of thin cloth in mesh gauze. The filtrate was then concentrated to dryness by exposure to air. Extracts obtained were scraped and stored in a refrigerator at 4℃ in labeled specimen bottles.

2.4 Collection and Laboratory Maintenance of Snail Host

Young *Bulinus* species (the intermediate host) were hand-picked from the field in Bauchi area and were maintained in glass aquarium. The young snails were fed ad-libitum with fresh lettuce.

2.5 Collection of Urine Specimen

Urine samples used for the work were collected from School children, ages 7-15 years from Miri Primary School (one of the most endemic areas of the disease) in Bauchi Local Government Area of Bauchi State, Nigeria. Samples were collected in wide mouthed glass bottles with screw cap tops. The urine samples were collected in the morning hours with the consent, help/backing and enlightenment by the school authority who accepted our proposal. The school was chosen because of the report and the endemic nature of the area with disease from previous research works [13,14].

2.6 Infection of the Snail

The snail, intermediate host (young *Bulinus species)* were infected by miracidia of *S. haematobium* according to the method of [15]. In the snail, miracidia are transformed into sporocysts given rise to the cercariae which escape from the snail and swim until the contact human skin [15,16].

2.7 Experimental Mice

Six weeks old laboratory mice (CBA strain) healthy and physically stable were selected from the animal house, University of Jos, Plateau State Nigeria. The mice were randomly divided into (A, B, C, D, E, F, G, H and I). Group A – H were used as the experimental animals while those in group I were used as the control. Meanwhile mice were maintained on balance mouse pellets.

After treatments, mice were sacrificed, each mouse was dissected to expose the gastro intestinal tract as well as the lungs, liver and the heart. The dissected mouse was clamped to the perfusion board with the aid of clips, stretching the animal well to expose the organs. The hepatic portal vein was completely severed to allow for the passage for worms during the process of perfusion as described by [17]. Tissues analyzed for egg count included the gastro-intestinal and associated organs (Liver, Spleen and pancreas).

3. EXPERIMENTAL PROCEDURE

3.1 Study Design

There were eight experimental cages. Two cages separately were used and the mice therein treated with the methanol extract. The first cage

selected for treatment had mice treated on day zero with methanol extract. The rest of the experimental cages were treated on days 7, 14, 21, 28, 35, 42 and 49 (Table 1). The extracts were applied at rate of 40 mg/kg (dose selected was determined after a pilot study); thus, mice were weighed individually to determine the amount of extract to administer. Doses for each mouse were dissolved in pre-determined volume of water (0.3 ml). The volume represents what a mouse would readily take after starving it of water for 24 hours. All treated mice were therefore starved of water prior to extract treatment. Positive control mice were not treated. The last cage was treated on the 49th day and after 56th day recording of data was started.

3.2 Infection of the Final Host

After the incubation period at 26°for 31 days, the infected snails were exposed to strong electric light in small containers, the shedding of the cercariae started. These cercariae with maximum survival time of 3 hours were used to infect the mice. The infection process was through a Danish Bilharziasis Laboratory procedure, where the mice were put into water containing 200 cercariae for 30 minutes [18].

3.3 Administration of Plant Extracts, (*Prosopis africana and Erythrina senegalensis*)

This was done using stomach tube method [17]. The extracts were administered orally with a rubber tube attached to a syringe.

There were eight experimental cages each containing five mice. Two cages separately were used and the mice therein treated with the methanol extract. The first cage selected for treatment had mice treated on day zero. The rest of the experimental cages were treated on days 7, 14, 21, 28, 35, 42 and 49 (Table 1). The extracts were applied at rate of 40 mg/kg; thus, mice were weighed individually to determine the amount of extract to administer. Doses for each mouse were dissolved in pre-determined volume of water (0.3 ml). The volume represents what a mouse would readily take after starving it of water for 24 hours. All treated mice were therefore starved of water prior to extract treatment. Positive control mice were not treated. The last cage was treated on the 49th day and after 56th day recording of data was started.

3.4 Efficacy of the Extracts

This was determined on the basis of percentage reduction in worm yield; percentage reduction in tissue egg count in treated mice compared with untreated/infected control. Method for quantifying the parameters is documented by [17] for reduction on worm yield and tissue egg count.

A student t-test was applied to determine the significance or otherwise of difference between corresponding mean values, the data were expressed as mean ± standard error of mean (SEM). The student t-test was used to compare means of treated groups and control.

3.5 Phytochemical Analysis

Phytochemical analysis consisting of performing simple chemical tests was carried out to detect the presence of tannins, anthroquinones, cardiac glycoside, saponins, carbohydrates, steroids and

Table 1. Mean recovery of adult worm from mice infected with cercariae and treated at weekly interval with *Prosopis africana*

Treatment schedule (Days after Infection)	Worm Burden	Percentage worm recovery	Percentage worm reduction	Students t-test
Infected/untreated control	52	26		
0	1	0.5	98	p<0.05
7	1	0.5	98	p<0.05
14	2	1	96	p<0.05
21	49	24.5	5.77	Ns
28	47	23.5	9.62	Ns
35	43	21.5	17.31	Ns
42	2	1	96	p<0.05
49	1	0.5	98	p<0.05

Mean for each treatment were compared with the control at p<0.05; Ns-Not significant

terpenes in the plant extract which contain active ingredients that shows anti-helminthic activity against the disease. The analysis was carried out on the distilled water extract following the procedure described by [10].

3.6 Test for Tannins

To 5 g of each portion of plant extract was stirred with 10 ml of distilled water, the resultant solution was filtered and ferric chloride reagent was added drop wise and the resultant reaction was noted.

3.7 Test for Anthraquinones

2 g of each plant extract was shaken with 4 ml of benzene, the solution was filtered and 4 ml of ammonia solution was added to be filtrate. The mixture was shaken and the resultant reaction was noted.

3.8 Test for Cardiac Glycosides (Salkoski Test)

0.5 g of each plant extract was shaken with 10 ml of distilled water in a test tube for 1 minute. It was kept in water bath and was observed for persistent frothing for 2 minutes. The resultant reaction was noted.

3.9 Test for Carbohydrates

A solution of each of the plant extract was dissolved in 5ml of distilled water and subjected to Molisch's test and the result noted.

3.10 Molisch's Test

Enough of 1-napthol was taken to cover the bottom of a test tube and this was dissolved in about 10 drop of ethanol. This was transferred to extract solution and then drops of concentrated sulphuric acid (H_2SO_4) were poured down the side of the tube to form a layer at the bottom of the ethanoic solution.

3.11 Test for Steroid and Terpenes

To 0.5 g of each of the plant extract was dissolved in 2 ml of chloroform and subjected to Lubermann-Burchard test and was observed for the presence of steroid and terpenes.

3.12 Statistical Analysis of Data

Results obtained were expressed as mean ± standard error of mean (SEM). The student t-test was used to compare means of treated groups and control for any significant difference in parasitaemia of mice treated with leaf extracts and the control group using the formula:

$$t = \frac{x - u}{s/\sqrt{n}}$$

4. RESULTS

Tables 1 and 2 show the mean worm recovery. The percentage worm reduction revealed that mice treated on days 0, 7, 14, 42 and 49 were highly susceptible to treatment with the plant extracts (*Prosopis africana and Eythrina senegalensis)* at the dose of 40 mg/kg. on the other hand, mice treated on days 21, 28 and 35 were not susceptible. The mice treated on days 0, 7, 14, 42 and 49 had significant declined in mean worm recovery compared to the control. Those treated on the other days had comparable mean worm recovery with the control. The percentage cure rate given by the percentage mean worm recovery (Tables 1 and 2) showed that treatment achieved 96-98% and 94-96% cure for *Prosopis africana* and *Erythrina senegalensis* extracts respectively, based on data for days 0, 7, 14, 42 and 49.

Tables 3 and 4 show the tissue egg distribution in different segment of the gastro-intestinal tract (GIT) and the associate organs. The percentage reduction in tissue egg count revealed that there was a remarkable decline in mean tissue egg for mice treated on days 0, 7, 14, 42 and 49 compared with the control. The total eggs for days 0, 7, 14, 42 and 49 differed significantly ($P<0.05$) from the control value. Cure as depicted by percentage reduction in mean tissue egg count for *Prosopis africana* and *Eythrina senegalensis* was up to 98% and 96% respectively.

Tissue egg count was considered for different segments of the GIT and associated organs (liver, spleen and pancreas). It was observed that egg counts were higher in the rectum than in the colon. The two segments in Tables 3 and 4 each accounted for 63.5% of the tissue eggs, the rest of the GIT, accounted for 29.47%, while the liver, spleen and pancreas accounted for 6.96%.

Visual examination of the liver and spleen of mice treated on days 0, 7 and 14 showed healthy organs comparable to those of healthy mice. On the other hand, both control mice treated on days 21, 28, 35, 42 and 49, showed visible indication of gross hepatosplenic disease and sandy patches.

Table 2. Mean recovery of adult worm from mice infected with cercariae and treated at weekly interval with *Erythrina senegalensis*

Treatment schedule (days after infection)	Worm burden	Percentage worm recovery	Percentage worm reduction	Students t-test
Infected/ untreated control	52	26		
0	3	1.5	94	p<0.05
7	3	1.5	94	p<0.05
14	2	1	96	p<0.05
21	46	23	11.55	Ns
28	48	24	7.7	Ns
35	41	20.5	21.2	Ns
42	3	1.5	94	p<0.05
49	2	1	96	p<0.05

Mean for each treatment were compared with the control at p<0.05; Ns-Not significant

Table 3. Mean tissue egg count of the gastro-intestinal tract and associated organs of mice treated at weekly interval after infection with the *Schistosoma cercariae*

Treatment with *Prosopis africana*							
Treatment schedule (days after infection)	**Liver spleen and pancreas**	**Oesophagus Stomach and duodenum**	**Colon**	**Rectum**	**Total no. of eggs**	**Percentage reduction over control**	**Students t-test**
Infected / untreated control	51	216	302	164	733	-	
0	3	4	10	19	36	95	p<0.05
7	7	3	2	9	21	97	p<0.05
14	3	5	11	20	39	9	p<0.05
21	40	204	210	264	718	2.05	Ns
28	42	202	230	251	728	1.1	Ns
35	37	229	187	258	711	3.0	Ns
42	2	3	5	7	17	98	p<0.05
49	4	7	12	43	66	91	p<0.05

Mean number of egg for each treatment was compared with the control at p<0.05;Ns-Not significant

Table 4. Mean tissue egg count of the gastro-intestinal tract and associated organs of mice treated at weekly interval after infection with the *Schistosoma cercariae*

Treatment with *Erythrina senegalensis*							
Treatment schedule (days after infection)	**Liver spleen and pancreas**	**Oesophagus stomach and duodenum**	**Colon**	**Rectum**	**Total no. of eggs**	**Percentage reduction over control**	**Kruskal wallis**
Infected / untreated control	51	216	302	164	733	-	
0	4	6	12	30	52	93	p<0.05
7	2	5	8	25	40	95	p<0.05
14	4	7	15	32	58	92	p<0.05
21	44	209	205	250	708	3014	Ns
28	42	275	99	253	669	8.73	Ns
35	48	283	104	222	657	10.37	Ns
42	2	7	7	11	27	96	p<0.05
49	8	3	4	13	28	96	p<0.05

Mean number of egg for each treatment was compared with the control at p<0.05; Ns-Not significant

Phytochemical screening of the plant extracts used revealed the presence of tannins, saponins, anthraquinones, cardiac -glycosides, carbohydrate and steroids, while Terpenes were absent (Table 5). These extracts showed anti-helminthic activity against the disease schistosomiasis.

4.1 The Efficacy Level

Looking at the percentage reduction in the mean tissue egg count, it was obvious that *Prosopis africana* and *Erythrina senegalensis* proved to be effective. The cure as noted by the percentage reduction showed that for *Prosopis africana* it was 96%-98% while for *Erythrina senegalensis* it was up to 96%. This shows that the efficacy level of *Prosopis africana* is slightly higher than that *of Erythrina senegalensis.*

Table 5 show active ingredients present in the plant extracts.

Pytochemical Screening on Distilled Water Extracts of *Prosopis africana and Erythrina senegalensis*

5. DISCUSSION

Doenhoff and Bain [19], and Sabah et al. [20] independently investigating the chemotherapy of schistosomiasis, using mouse models, had difficulty in determining exactly the stages at which the migratory phases of the disease were unaffected by treatment. Concerning the fact that the administration of anti-schistomal agents produces a temporary or definitive suppression of egg laying, the decrease or complete cessation of egg passing by treated animals. The practice used for the evaluation of therapeutic activity in schistosomiasis treatment (Pellegrino and Katz, 1973). The Low egg output by the experimental mice in this study indicated that the extracts of *P. africana* and *E. senegalensis* have some anti-schistosomal properties, thus, confirming the traditional use of the extracts. Doenhoff and Bain [19] observed that worms at 3, 4 and 5 weeks of post-infection were not susceptible to drug treatment, despite the fact that during this period they had reached the hepatic portal system and so were subjected to the same titre of the schistosomicide available against them and found to be effective on worms 6 and 7 weeks after infections.

Table 5. Test for Tannins, Anthroquinones, cardiac Glycosides, Saponins, Carbohydrates, Steroid and terpenes

Test	Plant	Observation	Inference
Tannins	*E. senegalensis*	A blue black coloured Precipitate was observed	Tannin-Present
	P. africana	A blue black coloured Precipitate was observed	Tannin-Present
Anthraquinones	*E. senegalensis*	A pink colour in the ammonical (lower) Phase was obtained	Anthraquinone-Present
	P. africana	A pink colour in the ammonical (lower) Phase was obtained	Anthraquinone-Present
Cardiac Glycoside	*E. senegalensis*	A Reddish brown steroidal Ring was observed	Cardiac-glycosides Present
	P. africana	A Reddish brown steroidal Ring was observed	Cardiac-glycosides Present
Saponins	*E. senegalensis*	Persistent frothing on heating was observed	Saponins-Present
	P. africana	Persistent frothing on heating was observed	Saponins-Present
Carbohydrates	*E. senegalensis*	At the bottom of the ethanoic Solution a purple colour was observed	Carbohydrate-Present
	P. africana	At the bottom of the ethanoic Solution a purple colour was observed	Carbohydrate-Present
Steroids and Terpenes	*E. senegalensis*	A buish green coloured Precipitate observed	Steroids-Present Terpenees-Absent
	P. africana	A buish green coloured Precipitate observed	Steroids-Present Terpenees-Absent

Sabah et al. [20] also performed a similar experiment using the Puerto-Rican strain of *S. mansoni* and observed that the drug used was effective against developmental stages at weeks 1, 2, 5 and 6 but not at 3 and 4 weeks of infection.

The result obtained in this study also revealed that the early migratory stages were susceptible to drug treatment with the plant extracts on day 0, 7, 14, 42 and 49 and that intermediary stages i.e. those treated on day 21, 38 and 35, were not. This report agrees with those of Doenhoff and Bain [19] but differ slightly from those of Sabah et al. [20]. The differences exist in the duration after infection when worms are not reponsive to extract. Whereas Sabah et al. [20] found the worms at week 3 and 4 not responsive to treatment, in this research work, worms were also not responsive 5 weeks after infection.

Some researchers cited in Sabah et al. [20] had independently tried to explain the abnormality existing in the response of development stages of schisotomiasis to drug treatment with no success. It is believed that clearer explanation must be based on proper understanding of the physiology of development stages, host and the mode of action of drug.

6. CONCLUSION

Apart from the side effects, resistance to the drug of choice, praziquantel is an inorganic drug whose continual excretion into the environment may be harmful; with current global concern about the safety of the environment, it would be advantageous to have drugs developed from organic sources especially those of plant origin. There have been a number of claims regarding the efficacy of some local indigenous plants in the treatment of schstosomiasis by traditional healers. Among such plant are the *Prosopis africana and Erythrina senegalensis.* As this plants were therefore assayed and their efficacy established. It is hoped that this will provide the basis for the development of an effective, safe and environment friendly treatment of schistosomiasis.

ETHICAL APPROVAL

The research work was carried out in the Laboratory of the Department of Zoology, the Federal University, Lafia, Nigeria; with the consent of the Dean of Faculty of Science (ID No is: A0/072.).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ejezie GC. The parasitic disease of school children in Lagos State Nigeria. Acta Tropica. 1981;38:79-84.
2. Cowper SG. A review of helminthiasis in West Region of Nigeria, with special reference to the Ibadan Region. West Africa-Medical Journal. 1967;16:3-11.
3. WHO: Schistosomiasis Division of Control of Tropical Disease; 1999. Available:http//wwwtulaedu/dmsander/www224/schess.html
4. Hickman CP, Roberts LS, Keen SL, Eisenhour DJ, Larson A, I'Aason H. Integrated principles of zoology. 2011;15: 301-302.
5. Wu Z, Bu K, Yuan L, Yang G, Zhu J, Liu Q. Factors contributing to re-infection with *Schistosoma japonicum* after treatment in the lake of China. Acta Tropica. 1993;56: 83-88.
6. King CH, Manmoud AF. Drugs, five year later praziquantel. Annals of Internal Medicine. 1989;110:29-296.
7. Johansen MV, Monrad J, Christensen NO, Lindbery R. Experimental Schistosoma bovis in goats. Pathological Consequences of Prazequantel Treatment Journal of Comparative Pathology. 1996;115:1-11.
8. Fallon PG, Doenhoff MJ. Drug-resistant schistosomiasis: Resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. American Journal of Tropical Medicine and Hygiene. 1994;51:83-88.
9. Ismail MM, Metwally A, Farghaly A, Bruce J, Jao LF, Bennet JL. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. American Journal of Tropical Medicine and Hygiene. 1996;55: 214–218.
10. Sofowora A. Medicinal plants and traditional medicine in Africa. John Wiley. Ibadan. 3rd edition. 1993;150–153.
11. Audu JA. Traditional herbal treatment in Bauchi, Nigeria traditional medical system. 1989;3(1):1-8.
12. Ibrahim MA, Nwude N, Ogunsusi RA, Aliu YO. Screening of West African plants for

antihelminic activity. International livestock Centre for Africa Bulletin. 1984;17:19-23.
13. Istifanus WA. Ecological and allozyme studies on fresh water snails in Bauchi, Nigeria with special emphasis on Schistosome intermediate hosts. Unpublished Ph. D Thesis Abubakar Tafawa Balewa University, Bauchi, Nigeria. 1995;185.
14. Istifanus WA, Mohammed A, Tal KM, Mohammed DM. Prevalence and intensity of schistosoma infection among primary school children in Bauchi State, Nigeria. Nigerian Journal of Parasitology. 1989; 9-11:55–59.
15. Webbe G, James C. The importation and maintenance of Schistosomes of human and veterinary importance: In isolation and maintenance of parasites in Vivo. 9th Symposium of the British society for Parasitology. Ed. Tayor AER, Muller R. Blackwill Scientific Publications Oxford. 1971;77-107.
16. Frandsen F. Host parasite relationship of *Bulinus forskalii* and *Schistosoma intercalatum*. J. Helminthol. 1975;49.
17. Christensen NO, Gotche G, Frandsen F. Parasitological techniques for use in routine laboratory maintenance of schistosomes, and for use in studies on epidemiology of human and bovine schistosomiasis. Danish Bilharziasis Laboratory Handbook. 1984;1-40.
18. Christensen N, Frandsen O, Nansen P. The effect of some environmental conditions and final host on parasite related factors on the penetration of *Schistosoma mansoni* cercariae into mice. Z. Parasitenkd. 1979;57:267-275.
19. Doenhoff MJ, Bain J. The immune dependence of Schistosomicidal chemotherapy: Relative lack of efficacy of an antimonial in *S. mansoni* infected mice deprived of T cell and demonstration of antiserum synergy. Clinical and Experimental Immunlogy. 1978;33:235-238.
20. Sabah AA, Fletcher C, Webbe G, Doenhoff M. *Schistosoma mansoni* chemotherapy of infections of different ages. Experimental Parastology. 1986;61:294-303.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/13583