Composition of Some Traditional Malaria Remedies and their Antiplasmodial Effects on (*Plasmodium berghei*)

Gbolahan Wahab Ayoade[1], Titus Adeniyi Olusi[2], Isiaka Adekunle Amoo[1], Gbolahan-Ayoade Elizabeth Eka-ete[2]

[1]Department of Chemistry, School of Sciences, Federal University of Technology, Akure, Ondo State, Nigeria
ayoadegbolahan@yahoo.com & adisamoo@yahoo.co.uk
[2]Department of Biology, School of Sciences, Federal University of Technology, Akure, Ondo State, Nigeria
speak2lizudoh@yahoo.com & olusi2000@yahoo.com

Abstract: This study is aimed to establish the pharmacological basis for the acclaimed antimalarial activities of the bark extracts of the three herbs. Aqueous extract of the herbs was obtained by cold maceration (72 hrs) while methanol herb extract was by soxhlet extraction. Phytochemical tests were carried out on the three herbs, the antiplasmodal activity of the aqueous extract of the herbs were screened by in-vivo model in mice infected with *Plasmodium berghei* using 4 days suppression test. The herb extracts were significant ($p>0.05$) but with varying levels of antiplasmodal activity, as *Trema orientalis* has the highest (85%) suppression activity followed by *Morinda lucida* (83%) and *Alstonia soonei* (80%). The phytochemical screening of the herb extracts revealed the presence of alkaloids, glycosides, proteins, flavonoids, steroids, terpenoids, saponins and tannin. The results suggest that the bark extracts of *A. soonei, M. lucida* and *T.orientalis* possess significant antimalarial activity.

Key words: Herbs extract, Phytochemical, *Plasmodium berghei*, antimalarial, suppression

1. Introduction

Malaria is a very old disease since *Anopheles* mosquito that transmits the disease is as old as mankind. The writings of the Samarians, Egyptians, Ancient Chinese, Homer, Aristotle, Plato and Shakespeare some dating 3,500 to 4,000 years ago, described intermittent fevers and splenomegaly, characteristic of malaria [1]. Malaria is believed to have originated from West Africa (*P. falciparum*), West and Central Africa (*P. vivax)* (on the basis of the presence of homozygous alleles for hemoglobin C and RBC duffy negativity that confer protection against *P. falciparum* and *P. vivax* respectively) and transported to other parts of the world through trans-pacific voyages, slave trade and man's migration [2]. He reported that man and malaria seemed to have evolved together, and that the ancestors of malaria parasite probably existed at least half a billion years ago. At present, about 100 countries or territories in the world are considered malarious almost half of which are in Africa, south of the Sahara. Although this number is considerably less than it was in the mid 1950s (140 countries or territories) more than 2400 million of the world's populations are still at risk [3]. The manifestation of the disease in different populations varies across the continent. In many parts of Africa, young children and pregnant women are most affected while in Asia adults as well as children are equally affected [4]. In Nigeria, there is an element of uniform distribution and prevalence of the disease in both rural and urban areas. This may be due to the vectors, *Anopheles gambiae* , *Anopheles arabiensis* and *Anopheles funestus* breeding in fairly clean sun lit water pools e.g. water storage tanks and wells which are found in both rural and urban areas [1]. Malaria in Nigeria is holoendemic i.e. there is an intense all-year round transmission with greater intensity in the wet season than dry season. The use of anti – malaria drugs and other means have not been fruitful over the years, however, the use of herbal medicine in combating malaria has been reported by many researchers as a good alternative, but little has been done in examine the chemical composition of these herbs. This study is therefore designed to examine the phytochemical composition of the three herbs used in order to establish its acclaimed antiplasmodial activity on the experimental animals (mice) infected with P. berghei.

2. Literature

Before the advent of orthodox medicine in the treatment of ailments which include malaria, the traditional African society had devised various means of combating such ailments. One of the major ailments that are of concern in the world today is malaria. Malaria is the single most important cause of ill health, death and poverty in the Sub-Saharan Africa. About 200 to 300 million new cases of malaria occur worldwide each year, and about one to three million deaths occur of which 2 / 5 occur in Africa [3]. The disease is believed to be a major obstruction to social and economic development in Africa, causing enormous misery and suffering through the pain of fevers and the anguish of bereavement. Ninety percent of the deaths caused by malaria occur in children aged less than five year old [5]. However, the problems militating against the effective management of malaria have been enumerated. The most important problem is that Plasmodial parasites are resistant to most widely available, affordable and safest first line treatments such as Chloroquine and Fansidar [6] or secondly , the overall control of mosquitoes which transmits malaria is made difficult by their resistance to a wide range of insecticides. The third which is a new and rapid developing problem is the wide production of fake

antimalarial drugs. In Southeast Asia 32% and 53% of artesunate blister packs sampled contained no active ingredients [7]. Lastly, most countries in Africa lack the necessary infrastructure and resources to manage and control malaria [5].

A number of traditional herbs have been tested and used in the prevention and also treatment of malaria including Artemisia annua, old leaves of Carica papaya, roots and leaves of *Guinensis*, unripe fruit of *Capsicum frutescence* and *Azadirachta indica* popularly called Dongoyaro in Nigeria [8]. Recent studies indicate that lemon grass can be successfully used to treat drug resistant malarial and typhoid fever [9]. It has been discovered that several drugs most of which are used for the treatment of malaria can be taken for prevention.

3. Previous work

A number of traditional herbs have been tested and used in the prevention and also treatment of malaria including Artemisia annua [8], old leaves of Carica papaya, roots and leaves of *Guinensis*, unripe fruit of *Capsicum frutescence* and *Azadirachta indica* popularly called Dangoyaro in Nigeria. Recent studies indicate that lemon grass can be successfully used to treat drug resistant malarial and typhoid fever [9]. It has been discovered that several drugs most of which are used for the treatment of malaria can be taken for prevention. In what is best described as an attestation to the efficacy of herbs in the prevention of malaria in the country, experts have found the water extracts of pawpaw and mango leaves showed potencies against malaria parasites and that they compare favourably with an established long acting orthodox anti-malarial drug, sulphadoxine/pyrimethamine. One of the common drugs in this group is Fanisidar (SP). *Carica papaya* or pawpaw has also been studied for its many medicinal uses. Its fruit rind is reportedly used in India by traditional healers for the treatment of 'recurrent fever'. On the other hand, the medicinal uses of the fruits, stem, bark, leaf and root of *Magnifera indica* (mango) have been recognized for ages.

4. Methods / Approach

4.1 Materials

4.1.1 Collection, Authentication and Preparation of plant material

Fresh leaves and bark of *A. soonei* ***(Afefe),*** *M. lucida* ***(Oruwo) and*** *T. orientalis* ***(Ahun)*** were collected from forest zone of the Federal University of Technology, Akure, Ondo State Nigeria**.** The plant leaves and bark were taken to the Department of Crop Soil and Pest Management (CSP) of the University for Clear Identification and they were carefully identified and labeled by curators. The samples were air dried and later pulverized to coarse powder using a laboratory milling machine (Model **4** Arthur Thomas, USA).

4.1.2 Malaria Parasite: *Plasmodium berghei* obtained from the Department of Parasitology. Institute of Medical Research and Training (IMRAT), University College Hospital (UCH) Ibadan, Nigeria was used.

4.2 Extraction and Fractionation of Plant Material: The plant materials were divided into three parts. One part was extracted with metllano1 using a Soxhlet extractor till the solvent became clear. Another part was soaked in water for 72hrs while the remaining parts were successively extracted with n-hexane using a soxhlet extractor. The extract and fractions were evaporated to a dry residue using a rotary evaporator (Rotavapour).

4.3. Sample Animal and weight determination Swiss albino mice used in this research were collected from the Institute of Medical Research and Training (IMRAT) University College Hospital UCH Ibadan, Nigeria, and then transferred to the laboratory in the animal section of the Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria. A total number of fourteen (14) mice were used for this research at equal number of male and female weighing between 17g and 28g and were fed on standard diets manufactured by Pfizer Livestock Feeds LTD, Nigeria. They were kept in separate cage (3.5cm x 6.5cm), same environmental conditions of 12 hours light and 12 hours darkness, normal room temperature of 27^0C were maintained for all the animals throughout the period of investigation and were not denied access to water.

4.4. Inoculation of the mice: Each of the Swiss albino mice was intraperitonally administered with a standard inoculums of P *berghei*. Stored parasitized blood in liquid Nitrogen was allowed to thaw. The content was injected into three donor mice and left for five days so as to allow for the development of parasitemia. The parasitized blood was collected from each donor mouse and diluted appropriately with Phosphate buffer saline to make 1×10^7 parasitized red blood cells, as described by [10]. 0.2ml of the diluted inoculums was then injected into each of the sample mouse after which they were left for three days. The aim was to achieve a high level of parasitaemia. However, thick blood smear were prepared from their tail veins and viewed under the × 40 microscope after five

days. The parasitaemia count was carried out by the use of a tally counter and the average percentage parasitaemia calculated using the approach of [11] as thus:

Average % Parasitaemia ═ No of parasitaemia / No of WBC × 100

4.5. Collection of peritoneal macrophage: All the cytological preparations were done under sterilized conditions. Peritoneal macrophages were collected from the sacrificed mice and washed in chilled PBS, pH 7.2 supplemented with 0.01M EDTA and 1% glucose by centrifugation. The macrophages were resuspended to 10^6 cells/ml in DMEM supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 mg /ml streptomycin.

4.6. Antiplasmodial Studies: Preliminary evaluation of the antimalarial activity of aqueous methanol extracts of the three herbs was conducted. Antiplasmodial activity of aqueous methanol extracts was screened by in-vivo model in mice infected with *plasmodium berghei* using 4-day suppression. A donor mouse heavily infected with parasites was anaesthetized using chloroform and the blood was collected through cardiac puncture. The presence of parasitemia was established by microscopic examination of a thin blood film. 1ml of the blood was diluted with normal saline to 20ml in which 0.2ml was injected intraperitoneally to healthy mice used for this research. The infected mice were later grouped into three separate groups of four animals each since three herb extracts was prepared. The animals were treated with extracts shortly after inoculation on day zero to avoid death. They were left for 4 days after which the thin film of tail veins blood of each mouse was made on a microscopic slide and viewed under the × 40 microscope. The film (smear) were stained with Leishman stain and examined under microscope. Parasitemia level was determined by counting average number of parasites in 10 fields of at least 1000 erythrocytes by the use of a tally counter and the average percentage parasitaemia calculated using the approach of [11] as thus:

Average % Parasitaemia ═ No of parasitaemia / No of WBC × 100.

Average % Suppression ═ Av. % Parasitaemia in control ─ Av. % Parasitaemia in treated /

Av. % Parasitaemia in Control × 100

The parasitized red cells were recorded as the percentage of total RBC's as against the control.

4.7 Phytochemical Screening

Basic phytochemical screening consists of performing simple chemical tests was used to detect the presence of alkaloids, tannins saponins, anthraquinones, phylobatannins and cardiac glycosides on plant extracts [12].

(i) Test for alkaloids:

About 0.1g of each extracts was stirred in 5ml of 1% aqueous hydrochloric acid in a steam bath, 1ml of the filtrate was treated with a few drops of dragendooffs reagent. Turbidity or precipitation was taken as the primary evidence for the presence of alkaloids in the extracts being evaluated [13].

(ii) Test for saponins

The ability of saponin to produce frothing in aqueous solution and to haemolyse red blood cell was used as screening test for these compounds. For the frothing test, the method described by [14]. About 1ml each of the extract was shaken with water in a test tube. Frothing which persist on warming was taken as preliminary for the presence of saponin.

(iii) Test for tannins

About 5.0g of each portion of plant extract was stirred with 10ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue – black green or blue – green precipitate was taken as evidence for the presence of tannins [13].

(iv) Test for cardiac glycosides

a. **Legal test** The extract was dissolved in pyridine and a few drops of 2% Sodium Nitroprusside together with a few drops of 20% NaOH were added. A deep red color, which faded to brownish yellow, indicated the presence of cardenolides.

b. Liberman's Test

About 0.5g of the extract was dissolved in 2.0ml of acetic anhydride and cooled well in ice. Sulphuric acid was carefully added. A color change from violet to blue to green indicated the presence of steroidal nucleus [14].

c. Salkowski Test About 0.5g of the extract was dissolved in 2.0ml chloroform. Sulphuric acid ($H_2SO_4$was added carefully added to form a lower layer. A reddish – brown color at the interface indicated the presence of steroidal ring and terpenoid.

d. Killer – Kiliani Test
About 0.5g of extract was dissolved in 2.0ml glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1.0ml concentrated Sulphuric acid. A brown ring obtained at the interface indicated the presence of deoxy sugar – a characteristic of cardenolides. [14].

(v) Test for carbohydrate

i. Molisch test for carbohydrates
20mg of plant extract was further extracted by warming with 5ml of H_20 in a water bath for 2min. To the cold aqueous extract (filtrate) was added a few drops of 2% w/v aqueous solution of a - naphthol. After mixing thoroughly, Iml of conc. H_2SO_4 was gently poured down the side of the test tube into the mixture. A positive result is indicated by the appearance of a brown ring at the interface.

(vi) **Fats and oils**
A small quantity of the extracts was pressed on paper; translucency indicates the presence of fats and oils.

(vii) **Test for Resins**
a. Precipitation test: About 30g of the extracts were further extracted with 5ml 96% ethanol. The resultant alcoholic extract was then poured into 20ml of distilled water in a beaker. A precipitate shows the presence of resins.
b. Co1nu1- Test: About 5mg of the extracts was further extracted with chloroform and the resultant extracts concentrated and evaporated to dryness. The residue was redissolved in 3m1 acetone and 3ml conc. HCI was added. The mixture was heated in a water bath for 30 minutes. A pink colour which changed to magenta red indicates the presence of resins.

(viii) Test for Protein
a. Millions Test: To a little portion of the filtrate in a test tube, two drops of Million's reagent were added. A white precipitate indicates the presence of proteins.
b. Xanthoproteic reaction test: About 5ml of the filtrate was heated with a few drops of concentrated nitric acid. A yellow colour which changes to orange with addition of an alkali indicates the presence of protein
c. Picric acid test: To a little portion of the filtrate was added a few drops of picric acid. A yellow precipitate indicates the presence of proteins.

5.0 Results and Discussion

5.1 Phytochemical Studies
The result of phytochemical screen carried out on the three herbs extracts is as presented in (table 1).

Table 1: Phytochemical Constituents of the Herb Extracts

Constituents	*A. Soonei (Afefe)*	*M. lucida (Oruwo)*	*T. orientalis (Ahun)*
Carbohydrate	++	+	++
Reducing sugar	+	-	-
Proteins	++	+	+
Glycosides	+++	+	++
Flavonoids	++	+++	+++
Steroids	+	+	+++
Terpenoids	+	+	++
Saponins	+	++	++
Tannins	++	++	++
Alkaloids	+++	++	+++
Fat and Oil	-	+	-
Resins	-	-	+

+++ = Abundantly present ++ = moderately present + = present - = absent

Alkaloids and glycosides were abundantly present in *A. Soonei*, while tannin, flavonoids, protein and carbohydrates are moderately present and the rest were present in minute except resins and oil. Only flavonoids was found to be abundant in *M. lucida* while alkaloids, tannins and saponins were moderately present and the rest were present in minute except resins and reducing sugar.

However, *T. orientalis* was found to be more abundant in alkaloids, steroids and flavonoids, while tannins, saponins, terpenoids, glycosides and carbohydrate were moderately present and the resins and protein were present in minute (trace) except oil and reducing sugar that are not detected. This implies that the three herb extracts were tested positive to almost all phytochemicals that are capable of protecting the body against foreign materials. These plant constituents have been associated with the anti-plasmodia1 effects observed in some plants [15]. Alkaloids are plant-derived compounds that are physiologically active, they contain one nitrogen in a heterocyclic ring, basic and have a complex structure and have been associated with anti-plasmodia1 activities [16]. One of the oldest antimalarial drugs, quinine is an alkaloid derived from the bark of cinchona plant. It is the principal anti-malarial compound found in cinchona bark [17]; [23]. Another alkaloid with anti-parasitic activity against *P. berghei* is naphthylisoquinoline [18]. Flavonoids have been shown to be the active constituents responsible for the anti-malarial effect of plants such as Zingiberaceae [15]. Qucrcetin is a flavonoid claimed to be responsible for the anti-malarial properties of *Azadracta indica* (Neem tree) popularly used as an anti-malarial agent in Nigeria [20]. It is also suspected to be present in *A. soonei and T. orientalis* and responsible for its anti-malarial properties [21].

Table 2: Percentage Parasitemia Count in Mice infected with *P. berghei*

Days / Mice	4	8	12	16	20	24	28
1	5	7	10	15	19	25	31
2	4	6	9	15	20	24	28
3	6	9	12	17	24	28	D
4	ND	5	8	12	16	21	29
5	4	7	11	16	21	25	32
6	5	8	13	18	23	26	32
7	3	6	9	13	19	24	31
8	7	10	14	20	26	D	D
9	5	7	9	15	19	24	30
10	ND	4	7	13	18	24	32
11	3	5	9	14	19	23	29
12	6	9	12	17	21	28	32
13	2	6	8	15	19	24	31
14	ND	ND	ND	ND	ND	ND	ND
Mean	4.54	6.84	10.07	15.38	20.30	24.67	30.63

ND = Not Detected D = Dead 13 = Infected control mice
14 = Non-infected control mice

5.2 Paracetemia

Table 2 presented the percentage infection cell in mice as determined at 4 days interval. It revealed that parasite development was not detected in two male mice at day 4 of infection together with non infected mouse (control), while the mean value of parasitemia in infected mice was 4.54% at day 4. It was observed that all infected has showed increased in parasitemia from the blood collected in day 8 of infection as indicated by average parasitemia of 6.84% but was not detected in non infected control. A significant increase ($P>0.05$) in parasitemia was obtained from day 12 to day 28 with average parasitemia of 10.07, 15.38, 20.30, 24.67 and 30.63% respectively, however, parasitemia was not detected throughout in non-infected control mouse but mortality was experienced at day 24 and 28 as one each mouse was found dead given a percentage mortality of 14.28% that 2 dead out of 14 experimental mice. Since the two dead mice were female, this implies that male mice were more tolerable than female when infected as they were more prolefirate.

Table 3: Suppressive Effects of *A.soonei* Extract on *P. berghei* Infected Mice

Days / *Mice*	4	8	12	16	20	Before treatment	Difference in parasitemia count	Parasitemia reduction (%)
1	29	23	18	12	D	31	D	D
2	24	20	16	10	6	28	22	78
3	D	-	-	-	-	-	-	-
4	24	19	14	9	5	29	24	82
Mean	25.7	20.7	14	10.3	5.5	28.5	23	-
Control	40.0	40.0	40.0	40.0	40.0	-	-	-
% Against Control	64.25	51.75	40.0	25.75	13.75	-	-	80

D = Dead C = Control = 40 (% infection)

Table 4: Suppressive Effects of *M. lucida* Extract on *P. berghei* Infected Mice

Days / Mice	4	8	12	16	20	Before treatment	Difference in parasitemia count	Parasitemia reduction (%)
5	30	24	20	12	D	32	D	D
6	31	D	D	D	D	32	D	D
7	27	21	17	10	5	31	26	83
8	D	-	-	-	-	-	-	-
Mean	29.3	22.5	18.5	11	5	31.5	26	-
Control	40.0	40.0	40.0	40.0	40.0	-	-	-
%Against Control	73.25	56.25	46.25	27.5	12.5	-	-	83

D = Dead C = Control = 40 (% infection)

Table 5: Suppressive Effects of *T. orientalis* Extract on *P. berghei* Infected Mice

Days / Mice	4	8	12	16	20	Before treatment	Difference in parasitemia count	Parasitemia reduction (%)
9	28	22	19	10	5	30	25	83
10	28	21	D	D	D	32	D	D
11	28	21	D	D	D	29	D	D
12	29	22	17	9	4	32	28	87
Mean	28.5	22	18	9.5	4.5	31	26.5	-
Control	40.0	40.0	40.0	40.0	40.0	-	-	-
%Against Control	71.25	55.0	45.0	23.75	11.25	-	-	85

D = Dead C = Control = 40 (% infection)

5.3 Suppressive Effect of the herbs

The percentage suppressive effects of the three herbs were presented in tables 3, 4 and 5 respectively. The average parasitemia suppression was recorded at 4 days interval after treatment of group of infected mice with herb extract. It shows that *Alstonias* had lower 25.7% parasitemia suppression than those treated with *M. Incida* 29.3% and *T. Orientalis* 28.5% as against 40% obtained for untreated control at day 4 of treatment. There was no significant difference in reduction level of parasitemia in the mice treated with *M. Incida* 22.5% and *T.orientalis* 22% which was higher than that of A. Soonei 20.7% at day 8 of herb administration and day 12 follows the same trend. However, the reverse was the case for day 16 and 20 where T. orientalis showed higher suppression (9.5 to 4.5) than M. Incida and A .Soonei with average parasitemia of (11 to 5%) and 10.3 to 5.5% respectively. The average percentage of parasitemia suppression for herb 3 in treated mice from day 4 to day 20 as against untreated control is given as: *A. Soonei* 64.25%, 51.75%, 40%, 25.75% and 13.75%; *M. Iucida*: 73.25%, 56.25%, 46.25% , 27.5% and 12.5% while *T. orientalis* gave 71.25%, 55%, 45%, 23.75% and 11.25% respectively. Generally, the overall percentage suppression of *T. orientalis* was (85%) higher than that of *M. Incida* (83%) and *A. soonei* (80%). Furthermore, the mortality rate in each group was also recorded during experimental period which showed that high mortality was recorded in group 2 (*M. Incida*) as 3 mice out of 4 were dead before the last day of experiment while 2 were found dead in each group of *A. soonei and T.orientalis* also, the control mouse dead before day 20 given a total of 7 and percentage mortality of 61.53% indicating that less than half of the infected experimental animal survived both infection and treatment to the end of the experiment. The result implies that the 3 herbs used for treatment of infected mice in this study has potency of reducing malaria parasite but *T. orientalis* known as (ahun) has the highest (85%) potency followed by *M. Incida* (83%) (Oruwo) and *A. soonei* (80%) (Afefe). The results obtained in this study were within the range of the results obtained by [22] as the mean % parasitaemia obtained in Group 1- 3 mice administered with methanolic, ethanolic and aqueous extracts of lemon grass were 43.01%, 50.21% and 48.08% while in Gp 4 - 6, 59.54%, 61.50% and 13.4% for the methanol, ethanol and aqueous extracts of neem respectively .The parasitaemia development in group 7 treated with standard drug (Malariech) was significantly minimal with 2.47s and 88.23% as% mean parasitaemia average suppression was recorded as against the highest (85%) that was obtained for *T.orientalis* in this study. Aqueous Neem extract exhibited the highest suppressive effect 76.21% followed by Lemon grass in respect of the methanolic (43.67%) and aqueous (38.07%) extracts as compared with methanolic (25.47%) and ethanolic (23.32%) extracts of Neem.

6. Conclusion

The study has revealed that the results of phytochemical test for the three herbs extracts (*A. soonei, M. lucida and T. orientalis*) showed positive to alkaloids, glycosides, proteins, flavonoids, steroids, terpenoids, saponins, and tannins indicating their presence in various proportions. All the herb extracts used for this research showed significant ($p>0.05$), but varying levels of antiplasmodal activity as *Trema orientalis* has the highest (85%) suppression activity followed by *Morinda Iucida* (83%) and *Alstonia soonei* (80%), This suggest that the bark extracts of the (*A. soonei, M. Iucida and T.orientalis*) possesses significant antimalarial activity. Since the three herbs extracts showed potency of reducing plasmodium, the study therefore suggest that the combination of three herbs should be adopted in treatment of malaria.

7. Future Scope

Further research work on the pharmacological study on average body requirement of herb extracts used in the treatment of malaria should be investigated and established.

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Author profile

Ayoade Gbolahan Wahab holds a Higher National Diploma in Biochemistry and a Post Graduate Diploma in Industrial Chemistry from the Federal University of Technology, Akure, Nigeria and currently undergoes a Master's degree in Analytical Chemistry in the same University. He is an Associate member of Nigerian Institute of Science Laboratory Technology, member Chemical Society of Nigeria, member Nigerian Society for Experimental Biology. His main area of research interest is Analytical/Environmental Chemistry.

Titus Adeniyi Olusi has a B.Sc. in Zoology, M.Sc. in Cellular Parasitology and PhD. in Applied Parasitology. He has over 29 years of teaching and research experience in tertiary institutions. He is a member and immediate past National Publicity Secretary of Parasitology and Public Health Society of Nigeria. He is a reviewer to several Local and International journals including *Innovation and Discovery*. He has examined several M.Sc. and Ph.D thesis for many Universities. He is a full fledge Professor of Parasitology in Department of Biology Federal University of Technology, Akure. He had published several journal articles in reputable on-shore and off-shore Institution and Society based Journals including the highly rated *Transactions of the Royal Society for Tropical Medicine and Hygiene, and Annals of Tropical Medicine and Parasitology*. The majority of his publications are well focused in the area of Parasitology (basic and applied) and his contribution to knowledge are mostly empirical information and baseline data on the nature and epidemiology of parasitic infections, which are vital in the information pool for basic, medical and veterinary sciences.

Gbolahan-Ayoade Elizabeth Eka-ete has a Higher National Diploma in Microbiology and a Post Graduate Diploma in Biology (Storage option) from the Federal University of Technology, Akure, Nigeria. She is an Associate member of Nigerian Institute of Science Laboratory Technology, member Parasitology and Public Health Society of Nigeria, member Nigerian Society for Experimental Biology. Her area of research focus is parasitology.

Isiaka Adekunle Amoo has a B.Sc. in Industrial Chemistry, M.Sc. Analytical Chemistry and PhD. Analytical Chemistry. He has acquired over 28 years of teaching and research experience in tertiary institutions. He is a member of Chemical Society of Nigeria, Institute of Public Analysts of Nigeria, Institute of Chartered Chemist of Nigeria, Science Association of Nigeria, Solar Energy of Nigeria and Nigerian Institute of Science Laboratory Technology. He is a full time Professor of in the Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria. He has graduated over 33 M. Tech, 9 PhD students and he has over 92 publications in reputable journals, both locally and internationally. He is the Editor-in-Chief of the journal of Laboratory Science, a publication of Nigerian Institute of Science Laboratory Technology, Federal Ministry of Science and Technology, Nigeria. Is a Professorial Assessor and External Examiner to many Universities such as; University of Benin, Benin City, Nigeria, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, Fountain University, Osogbo, Nigeria, University of Western Cape in South Africa, etc. He is a member of Nigerian University Commission Accreditation Team and also a member of Review Panel on Environmental Impact Assessment of Federal Ministry of Environment, Nigeria. He is the President, Conference of Heads of Chemistry Departments in West Africa. He is happily married with children.