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IN VIVO ANTIMALARIAL ACTIVITIES OF FRACTIONATED EXTRACTS OF *Asparagus africanus* IN MICE INFECTED WITH *Plasmodium Berghei*

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Abstract

This study was carried out to validate the traditional usage of *Asparagus africanus* for treating malaria in the traditional health care system of Ethiopia. The *in vivo* antimalarial activity of chloroform, butanol and aqueous fractionates (100, 200 and 300 mg/kg) of *Asparagus africanus* roots against a chloroquine (CQ) sensitive strain of *Plasmodium berghei* ANKA was assessed using the 4-day suppressive test procedure. The oral administration of the three fractions showed significant ($P<0.05$) parasitaemia suppression at all dose levels in dose related manner compared with the negative control. The butanol fraction showed the highest (85.94%) parasitaemia suppression at dose of 300 mg/kg/day while the aqueous residue induced parasitaemia suppression of 66.79% at the same dose. The chloroform fraction also showed significant parasitaemia suppression at all orally administered dose levels. The butanol fraction significantly prolonged ($P<0.05$) the survival times of *P. berghei* infected mice at the highest dose. The chloroform fraction and the aqueous residue at all doses significantly prevented ($P<0.05$) weight loss of infected mice that is commonly observed with increasing parasitaemia, whereas the highest dose (300mg/kg/day) of the butanol fraction failed to prevent weight loss. Preliminary phytochemical screening of the root powder of the plant indicated the presence of saponins, polyphenols, tannins and phytosteroids. The results of this study confirm the traditional usage of the plant for treatment of malaria. However, the active responsible principles are yet to be identified, which need further studies to elucidate the antimalarial mechanism of their action.

Key words: Anti-malarial activity, *Asparagus africanus*, Parasitaemia, *Plasmodium berghei*

Introduction

Malaria, one of the oldest human diseases, became the main concern of the World Health Organization (WHO) in the past few decades not only as a result of its re-emergence as the biggest infectious killer but also expansion of its distribution to previously non-affected areas. In Ethiopia, for example, the lowlands have always been regarded as areas of high malaria transmission; however, this appears to be changing due to climatic and ecological changes. Recently the epidemic is spreading into the highland areas where a large proportion of the population lives [1].

The resurgence of the disease due to drug resistant strains of the parasite and insecticide resistant strains of the mosquito vector catches the attention of many scholars. As a result of which, there has been various efforts to combat the problem of parasite resistance, including reversing chloroquine resistance; use of combination therapy; and discovery of new antimalarial compounds from various sources mainly from traditional medicinal plants [2,3].

Asparagus africanus (Lam) is traditionally used in treating various human ailments in Ethiopia including impotence, wound, diarrhea and Malaria [4-8]. It belongs to the Family Asparagaceae which includes 300 species in the genus *Asparagus*, widely distributed throughout Africa, parts of Europe, Asia and Australia. It is a perennial shrub or climber with stems up to 6m high growing between 700 and 3800m above sea level [9].

A range of medicinal plants with antimalarial properties including *Asparagus africanus* has been widely used by the traditional healers in Ethiopia. However, the effectiveness of most of antimalarial traditional medicines has not been scientifically evaluated. This study is therefore intended to investigate the antimalarial properties of the most highly practiced medicinal plants, *Asparagus africanus* (Lam), in the traditional health care system in Ethiopia.

Materials and Methods

Collection and Identification of plant material

The roots of *Asparagus africanus* were collected from the natural habitat around Meki area, 130km south of Addis Ababa. Identification of the plant was performed by plant taxonomists and a Voucher specimen (No. YA 24/01) was deposited at the National Herbarium, Addis Ababa University (AAU).

Extraction and Fractionation of Plant Material

Roots of *A. africanus* were cleaned and air dried under the shade in the Biomedical Laboratory, Department of Biology, Faculty of Science, AAU. The air-dried roots were then powdered by using an electrical grinding mill (Straub, model 4E, Philadelphia, USA); 400g of powder plant material was then macerated in 80% methanol and placed on orbital shaker (rotating 120 rpm). The extract was then filtered with Whatman filter paper N° 3 and the filtrate was dried using Rotary evaporator (BUCHI type TRE121, Switzerland). The aqueous residue was further lyophilized (Vacuubrad, GMBH Germany) to yield gummy residue (36g).

The Crude extract was subjected to further fractionation using three solvent systems (n-hexane, chloroform and n-butanol). 20g of the crude hydro alcoholic extract was suspended in separatory funnel in a 120 ml of distilled water and partitioned with 3 ×100 ml n-hexane at 40-60 °C. The n-hexane partitions were combined and labeled as Hexane fraction **(HF)**. The aqueous residue was then partitioned with 3 ×100 ml chloroform. The chloroform filtrates were combined and evaporated to give 0.85g of Chloroform fraction **(CF).** The aqueous residue was further partitioned with 3×100 ml n-butanol; the n-butanol fractionates were then combined, concentrated and labeled as Butanol fraction **(BF)** of 2.85g yield. The remaining aqueous residue was lyophilized to dryness and labeled as aqueous residue **(AF)** yielding 8g.

All the fractions except HF were kept in tightly closed container until used for *in vivo* testing. The

HF was discarded since it was very oily and difficult to dissolve.

Laboratory animal preparation

Healthy, adult male Swiss albino mice weighing 20-29g maintained in the Animal House of the Department of Biology, AAU were used in this study. All animals were housed in standard cages in the Animal House and fed a standard pellet diet and tap water *ad- libitum.*

The parasite strain

The antimalarial activity of the fractionated extracts of *Asparagus africanus* was tested using infected mice with CQ sensitive *Plasmodium berghei* strain ANKA maintained at the Animal House of the Department of Biology, AAU. The parasites were maintained by serial passage from infected mice to non-infected ones on weekly basis to keep the variability of the strain.

Phytochemical screening

Preliminary qualitative screening of major secondary metabolites of the root powder of *A. africanus* was conducted. The method was based on chemical tests involving color changes through reaction with different standard reagents [10].

In vivo antimalarial test

Antiplasmodial activities of the test fractions were performed in a 4-days suppressive standard test [11]. An infected blood sample was collected from the heart of a donor mouse with a rising parasitaemia of about 30%. The blood was diluted with normal saline in the intention that each 0.2 ml contained approximately 1×10^7 *Plasmodium berghei* parasitized erythrocytes. Male Swiss albino mice weighing 21-29 grams were inoculated on the first day (D_0), intraperitoneally, with 0.2 ml of infected blood. The mice were then divided randomly into five groups of six mice each for each fraction. The three groups of animals were assigned as the test groups whereas the other two groups were used as control (positive and negative). Three hours after infection, the three test groups were orally administered, with 100, 200 and 300 mg/kg/day doses of the fractionated root extract. Chloroquine at the dose of 10mg/kg/day and an equivalent volume of vehicle were administered to the positive and negative control groups, respectively, for four consecutive days (D_0 to D_3). This was done for each fraction of the extracts. On the fifth day (D_4), thin smears were made from the tail blood of each mouse, fixed with methanol and stained with 10% Geimsa. The parasitaemia level was determined by counting the number of parasitized erythrocytes out of 100 erythrocytes in random fields of the microscope.

Average percentage parasitaemia was calculated using the formula:

$$P = I_{rbc} / (I_{rbc} + NI_{rbc})$$

P = % Parasitaemia
I_{rbc} = Number of infected RBCs
NI_{rbc} = Number of non-infected RBCs

Average percentage of suppression was calculated using the formula:

$$S = (P_{nc} - P_{tg}) / P_{nc}$$

S = % Suppression
P_{nc} = Parasitaemia in negative control
P_{tg} Parasitaemia in test group

The body weights of the mice were taken to observe whether the test fractions of *A. africanus* roots prevented the weight loss that is commonly observed with increasing parasitaemia in infected mice. The weights were taken on D_0 and D_4.

Statistical analysis

The results were presented as the Mean ± SEM for each group of experiments. Data on parasitaemia, body weight and survival times were analyzed using windows SPSS Version 16. Statistical significance was determined by one-way ANOVA, the

Student's t-test and independent comparison tests. All data were analyzed at a 95% confidence interval (α=0.05).

Result

Phytochemical screening of the root powder of *A. africanus* revealed the presence of compounds including steroidal saponins, polyphenols, tannins and phytosteroids when screened using color forming and precipitating chemical reagents.

The *in vivo* antiplasmodial activity study revealed that the BF of the roots of *A. africanus* produced the highest chemosuppression in a dose dependent manner as compared to the CF and AF employed in this study. The chemosuppression was 47.24%, 59.03% and 85.94% for 100, 200, 300 mg/kg/day doses, respectively (Table 1). The chemosuppressive effect produced by all the test fractions was significant ($P<0.05$) compared with the negative control.

Comparison of the mean survival time of mice in the experimental groups with the untreated group for all test fractions was performed (Table 2). The result indicated that mice treated with 300mg/kg/day of the BF lived longer ($P<0.05$) than that of the negative control. All the test fractions employed in this study, except the highest dose of the BF, significantly prevented weight loss of mice at all dose levels ($P<0.05$) compared with the control group (Table 3).

see Table 1.

see Table 2.

see Table 3.

Discussion

The results have clearly showed that the different extracts of the root of *A. africanus* contain steroidal saponins, polyphenols, tannins, flavinoids, and phytosteroids. The therapeutic values of the plant may rely on some of the above chemical substances, particularly steroidal saponins, polyphenols and tannins that produce a definite physiological action on the human body [12, 13]. Various previous studies reported that phytocemicals like alkaloids, terpenoids, saponins, flavinoids and tannins are responsible for antiplasmodial activities of different plants. Some of these metabolites were also isolated from *Artemisia annua* and *Cinchona* Spp [14, 15].

In the present work the *in vivo* antiplasmodial experiments indicated that the test fractions showed from low to high parasitaemia suppression on day 4 post infection in the range 24.29%-85.94%. The highest being the BF at the dose of 300mg/kg/day while the lowest was recorded for the AF at the dose of 100mg/kg/day. This result is in agreement with a previous study for a water extract of roots of *A. africanus* employed against four different strains of *P. falciparum in vitro*. The study revealed the isolation of two compounds, a new steroidal sapogenin, which was named muzanzagenin, and (+)-nyasol through bioactivity-guided fractionation from the root of the plant that displayed moderate antiparasitic activity against *Leishmania major* and *P. falciparum in vitro* [16]. Therefore, the *in vivo* study is in good conformity to the previous *in vitro* study.

All the test fractions, except the highest dose of the BF, significantly prevented weight loss. The BF was able to prevent the expected body weight loss in a manner that the highest weight loss prevention was recorded by the lowest dose, whereas, the lowest prevention was by the highest dose of the fraction. The percent preventive effect declines with increasing dose which is in agreement with the results obtained on the crude extract of the same plant from prior study [17]. This decline might be a result of reduced feeding capability of mice treated with the highest dose of the fraction which might have an appetite suppressive effects escalating with increasing dose. This work did not, however, investigate this issue further.

Although not significant, the mice treated with the CF survived for longer periods than the mice in the negative control. However, the survival times declined with increasing dose concentration of the fraction. This might be explained by mechanisms of

drug actions such as having an indirect effect on the immune system, or by other pathways that are not understood in this study [18].

Conclusion

In this study the possible antimalarial activity of aqueous, chloroform and butanol fractions of *Asparagus africanus* roots was demonstrated against *Plasmodium berghei* in animal model. The butanol fraction was found to be the most potent fraction not only in its capability to suppress parasitaemia significantly but also in prolonging the mean survival of mice. Hence, the findings of this study confirm the traditional usage of the plant to combat malaria in Ethiopian folk medicine.

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Test fraction	Dose (Mg/kg/day)	% Parasitaemia ± SEM	% Suppression
Butanol	100	16.06 ± 0.41	47.24
	200	12.47 ± 0.49	59.03
	300	4.28 ± 0.39	85.94
	NC	30.44 ± 0.59	00.00
	CQ (10mg/kg)	00.00	100.00
Aqueous	100	25.65 ± 0.74	24.29
	200	19.62 ± 1.56	42.09
	300	11.25 ± 0.72	66.79
	NC	33.88 ± 1.54	00.00
	CQ (10mg/kg)	00.00	100.00
Chloroform	100	24.66 ± 0.60	25.34
	200	20.19 ± 0.96	38.07
	300	14.02 ± 1.32	57.55
	NC	33.03 ± 2.37	00
	CQ (10mg/kg)	00.00	100.00

Table 1: Antimalarial activities of different fractions of *Asparagus africanus* against *Plasmodium berghei* in Swiss albino mice

NC - Negative Control
CQ – Chloroquine

Test Fraction	Dose (Mg/kg/day)	Mean survival days
Butanol	100	8.83 ± 0.31
	200	9.5 ± 0.56
	300	11 ± 0.68
	NC	7.83 ± 0.31
Chloroform	100	9.33 ± 0.49
	200	9.00 ± 0.36
	300	8.66 ± 0.51
	NC	8.00 ± 0.37
Aqueous	100	9.83 ± 0.80
	200	8.83 ± 1.01
	300	10.83 ± 0.79
	NC	7.5 ± 0.56

Table 2: The effect of the test fractions of *A. africanus* roots on survival time of mice infected with *P. berghei*.

NC= Negative Control

Test fraction	Dose (Mg/kg/day)	Average weight before treatment (g)	Average weight after treatment (g)	%change
Butanol	100	24.42 ± 0.30	27.20 ± 0.55	11.40
	200	21.85 ± 0.22	23.28 ± 1.40	6.56
	300	25.5 ± 0.29	26.13 ± 0.79	2.48
	NC	23.33 ± 0.31	21.50 ± 0.58	-7.85
	CQ (10mg/kg)	21.25 ± 0.38	22.43 ± 0.58	5.57
Chloroform	100	22.50 ± 0.15	24.82 ± 0.53	10.31
	200	22.86 ± 0.24	25.18 ± 0.54	10.15
	300	22.37 ± 0.15	24.44 ± 0.31	8.94
	NC	23.25 ± 0.53	22.27± 0.36	-4.22
	CQ (10mg/kg)	27.91 ± 0.36	29.03 ± 0.66	4.01
Aqueous	100	22.75 ± 0.15	25.47 ± 0.39	11.94
	200	23.88 ± 0.22	24.93 ± 0.50	4.40
	300	27.5 ± 0.18	28.73 ± 0.34	4.48
	NC	21.50 ± 0.47	20.33 ± 0.54	-5.43
	CQ (10mg/kg)	26.63 ± 0.32	27.93 ± 0.33	4.88

Table 3: Body weight of *Plasmodium berghei* infected mice before and after the administration of the test fractions of roots of *Asparagus africanus*.

NC - Negative Control
CQ – Chloroquine