

Phytochemical Screening and Anti-malaria/Typhoid Fever Activities of *Alstonia boonei* (De Wild) Stem Bark Powder

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ABSTRACT

Anti-malaria/typhoid fever potential of graded doses of ethanolic and aqueous extracts of *Alstonia boonei* bark stem powder were investigated. The stem bark powder was also screened for the presence of some phytochemicals. The percentage chemo-suppressive activity (on early malaria infection in mice) of 100, 200 and 400 mg/kg body weight doses were found to be 81, 85 and 75%, respectively for the ethanolic extract and 56, 78 and 80%, respectively for the aqueous extract. This is substantial when compared to the 97% chemo-suppressive effect produced by 5 mg/kg body weight of chloroquine. The extracts did not produce any observable activity against *Salmonella typhi*. The phytochemical screening revealed the presence of saponin, flavonoids, terpenes, sterol and resins in the stem bark powder. These results further confirmed earlier reports that *A. boonei* possesses antimalarial activities.

Keywords: anti-malaria, anti-typhoid, phytochemical, *Plasmodium berghei*, *Salmonella typhi*

INTRODUCTION

Malaria, and recently typhoid fever are important causes of death and illness in children and adults in tropical countries. The predominant species of pathogenic organisms for malaria and typhoid fever are *Plasmodium falciparum* (Ukaga *et al.* 2006) and *Salmonella typhi* (HA, CCID 2003) respectively. The annual worldwide incidence of typhoid fever was estimated at 17 million cases, with 600,000 deaths (HA, CCID 2003). The mortality rate of malaria, estimated at over a million people per year had risen in recent years probably due to increasing resistance to anti-malaria medicines (WHO 2006). This latest information had led researchers to look for alternative anti-malaria therapy. One of such recourse is the investigation of medicinal plants. The medicinal value of these plants lies in their possession of a wide variety of physiologically active substances – the phytochemicals (Cowan 1999). An ethnobotanical survey of antimalarial plants used in some parts of Southwest Nigeria has been documented (Odugbemi *et al.* 2007; Idowu *et al.* 2010).

Alstonia boonei, a large evergreen tree belonging to the family Apocynaceae, is one of the widely used medicinal plants in Africa and beyond. It was named after Dr. C. Alston (1685-1760), a Professor of botany at Edinburgh University (Taiwo *et al.* 1998). The plant grows up to 45 m tall, the bole branchless for up to 25 m; the inner bark creamy or pale yellow with copious latex. It is distributed throughout the tropics and the rain forest of West and Central Africa (Oliver-Bever 1986; Olajide 2000) and is known by different names in different countries and tribal settings. These include; *Alstonia*, cheese wood, Patternwood (English); *Egbu* (Igbo); *Awuri* (Yoruba); *Mubajan-galabi* (Uganda).

Various documented and undocumented claims have it that alcoholic and aqueous preparations from some parts of the plant especially the stem bark have medicinal uses for treating febrile illness, micturation, jaundice, painful micturation, rheumatic conditions, etc. (Ojewole 1984; Asuzu and Onaga 1991). The stem bark of the plant is listed in the African Pharmacopoea as an antimalaria drug (Olajide *et al.*

2000).

This study is therefore aimed at screening the *A. boonei* stem bark powder for phytochemicals as well as verifying the antimalaria/typhoid fever activities of its ethanolic and aqueous extracts.

MATERIALS AND METHODS

Plant materials

The plant, *A. boonei* was collected from Olokwu, Emebiam, in Owerri, Nigeria. It was identified and authenticated by Mr. Ibe, the Chief Habarium, Department of Forestry, Michael Okpara University of Agriculture, Umudike, Nigeria. The stem bark sample was collected by scrapping using a blunt knife.

Chemicals and reagents

Giemsa stain was purchased from Tharma GMBH (Walsolmb, Germany). Mannitol salt agar was purchased from Neogen Corp. (Lansing, MI, USA). All other chemicals and reagents are of analytical grade.

Animals

50 Swiss albino mice weighing between 10-22 g were obtained from the animal house, Department of Pharmacology and Toxicology, National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja Nigeria, and used in the experiment.

Parasites

The *Plasmodium berghei* used was also obtained from NIPRD, Abuja. A standard inoculum of 1×10^7 of parasitized erythrocytes obtained from a donor mouse through the bleeding of the animal's caudal vein was in the value of 0.2 ml to infect the experimental animals intraperitoneally.

Test for anti-malaria activity

Makinde *et al.* (1989) version of the Suppressive Anti-malaria Test,

also called "Test on Early Malaria Infection" as reported by Maje (2007) was used in this study. For the ethanolic and aqueous extracts (separately), 25 mice were divided into 5 groups of five mice each. The animals were inoculated with the parasite at the beginning of the experiment (day 10). Three hours after inoculation (infestation 0 groups 1-3 mice were respectively given 100, 200, and 400 mg extract/kg body weight dose orally, group 4 animals, which served as positive control were given 5 mg chloroquine/kg body weight while the group five animals (negative control) were given 0.2 ml distilled water. The treatment was given once per day for four days. On the 5th day, two drops of the animals caudal vein blood samples were taken and transferred on slides – making thin film from each mouse. The slides were stained with Giemsa stain and subjected to microscopy. The average percentage parasitaemia and hence percentage chemo-suppression were evaluated for each of the does using the formulae:

$$\% \text{ Suppression} = (\text{Average parasitaemia in negative control} - \text{Average parasitaemia (in test dose)}/\text{Average parasitaemia in negative control}) \times 100$$

Anti-microbial screening (anti-typhoid fever studies)

The anti-microbial activities of the extracts against *Salmonella enterica serova typhi* were screened using Agar Diffusion method (Bonev *et al.* 2008). Briefly, the organism, *Salmonella enterica serova typhi* was isolated from stock culture using mannitol salt agar. With a swab stick, *Salmonella* was collected from the mannitol salt agar, spread a molten nutrient agar in an agar plate and allowed to dry. Then a hole was bored on each of the agar culture using a cork borer and the base of the hole sealed with a drop of the agar. A 2 mg/ml conc. of each of the extracts was introduced into separate holes with a sterile pipette and the cultures incubated without inverting the Petri-dishes at 37°C for 24 h. The cultures were observed for clear zone of inhibition. One of the cultures was incubated with 2 mg/ml peflacin (standard anti-*Salmonella typhi* drug) as control.

Phytochemical screening of *Alstonia boonei* stem bark powder

10 g of the stock sample was placed in 200 ml conical flask,

enough distilled water added to cover the sample and left to stand for 24 h with occasional stirring. The infusion was filtered using Whatman no.1 filter paper, and the water extract was collected and used for phytochemical tests. Tests for saponin and flavonoids were carried out following the method of Harborne (1983). Tests for tannin, alkaloids, cynogenic glycosides and anthraquinone were done according to the method of Evans (1989), while terpenes and sterols were tested using the method of Sofowa (1993).

RESULTS AND DISCUSSION

Table 1 shows the effects of ethanolic extract of *A. boonei* stem bark powder on early malaria infection in mice. The *in-vivo* evaluation revealed that the average percentage suppression of parasitaemia by the extract was 81, 85 and 75% at doses of 100, 200 and 400 mg/kg body weight per day, respectively. **Table 2**, on the other hand, portrays the effect of the aqueous extract on the same early malaria infection. From the table, the average percentage suppression of parasitaemia was 56, 78 and 80% at doses of 100, 200 and 400 mg/kg/day, respectively. Chloroquine, under the same experimental condition, at 5 mg/kg body weight per day produced chemo-suppression of 97%.

These results, in agreement with Ojowole (1984) and Olajide *et al.* (2000), show that the plant extracts possess antimalaria effect. Iyiola *et al.* (2011) also reported that ethanolic stem bark extract of *A. boonei* possesses potent antimalarial effect. Bello *et al.* (2009) reported the inhibited growth of *Plasmodium falciparum* using crude stem ethanolic extract of *A. boonei*. While the chemo-suppressive effect of aqueous extract was dose-insensitive, the ethanolic extract exhibited a progressive dose-response relationship. The highest chemo-suppression (80%) observed at the highest dose (400) mg/kg/day for aqueous extract is lower than the highest value (85%) observed at medium dose (200 mg/kg/day) for ethanolic extract. This may be considered as an indication that the potency of ethanolic extract in the suppression of parasitaemia is more than that of aqueous extract. Relative to the value of 97% obtained for chloroquine (an established anti-malaria drug), ethanolic extract at 200 mg/kg/day effectively suppresses parasitaemia.

The result of microbial screening indicated that both ex-

Table 1 Effects of ethanolic extract of *Alstonia boonei* stem bark powder on early malaria infection in mice.

Treatment	Dose (mg/kg/day)	Average parasitaemia*	Percentage suppression
Extract	100	10.20 ± 0.86	81
Extract	200	8.00 ± 0.77	85
Extract	400	13.75 ± 3.12	75
Chloroquine	5	1.60 ± 0.24	97
Distilled water	0.2 ml	54.40 ± 4.45	0

*Values were presented as mean ± SEM, n = 4

Table 2 Effects of aqueous extract of *Alstonia boonei* stem bark powder on early malaria infection in mice.

Treatment	Dose (mg/kg/day)	Average parasitaemia*	Percentage suppression
Extract	100	25.00 ± 0.5	56
Extract	200	12.20 ± 2.25	78
Extract	400	11.50 ± 2.72	80
Chloroquine	5	1.60 ± 0.24	97
Distilled water	0.2 ml	54.40 ± 4.45	0

*Values were presented as mean ± SEM, n = 4

Table 3 Result of phytochemical screening of *Alstonia boonei* stem bark powder.

Test	Remark
Alkaloids	-
Anthraquinones	-
Flavonoids	+
Cynogenic glycoside	-
Resins	+
Saponins	+
Sterols	+
Tannins	-
Terpenes	+

Key: + = Present; - = Absent

tracts of the test sample showed no activity against *Salmonella typhi* relative to a standard anti-typhoid fever drug (Peflacin) that produced a 10mm (mean diameter) zone of inhibition under the experimental condition. This non-sensitivity of the test organism to the extracts is at variance with the general contention (claims of folk medicine) that the plant extract was used in the treatment of typhoid fever.

The phytochemical screening shows that *A. boonei* stem bark powder contains flavonoids, resins, saponins, sterols and terpenes, while alkaloids, anthraquinones, glycosides and tannins were absent (**Table 3**). Fasola and Egunyomi (2005) had earlier identified saponins, alkaloids, tannins and cardiac glycosides as the major phytochemicals in the stem bark of *A. boonei*.

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