Original Research Article

Prophylactic, Curative and SuppressivePhyto-Medicinal Therapy of Synergistic Efficacy of *AlstoniaBoonei*&*Capsicum Frutescens* Extract against Plasmodium*Berghei*(NK 65)/*Salmonella Typhi*(ATCC 35723) InfectedSwiss Albino Mice

Abstract

The purpose of this research work is to evaluates the potential medicinal activity of ***Alstoniaboonei and*** *Capsicum frutescens* extracts for Prophylactic, Curative & Suppressive phytomedicinal therapy against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice**.**Alstonia*boonei*belongs to the Apocynaceae family has severally been reported to have medicinal properties, for curing various diseases while Capsicum is a tropical and an important agricultural crop, the popular vegetables, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit**,** Stem bark (Alstoniaboonei) and *Capsicum frutescens* fruit were collected from AdekunleAjasin University's reserve forest. Both plant samples were authenticated in the herbarium unit of Forest Research Institute of Nigeria (FRIN) with identification number FHI 109806,109872 respectively.Male Swiss albino mice weighing between 18-20g were obtained from the animal house, as well as the *Plasmodium berghei*(NK 65) and *Salmonella Typhi*(ATCC 35723) were also obtained from Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed equally on rat chow and water throughout the period of the experiment. A standard inoculum of 1×10^{7} of parasitized erythrocytes from a donor mouse and$1x10^{-6}$innoculum size of Salmonella typhi in volumes of 0.2 ml were used to infect the experimental animals intra-peritoneally.The suppressive antimalarialtestalso called"Test on Early Malaria Infection" was used in this study.Twenty five Swiss albino mice were divided into five groups of five mice each which were inoculated with the parasite and the organism (*Plasmodium berghei*/*Salmonella typhi*) on the first day of the experiment (day 1) The mice were not treated until the parasitaemia was established. On day 4 i.e. 72 hours after the animals were infected. Group's 1-3 mice received 100, 200 and 400mg/kg body

weight of the extract per day orally. While the 4th group which served as the positive control received 10mg/kg chloroquine/Ciprofloxacin orally, mice in 5th group received 0.2ml distilled water and served as negative control for the same period. Each of these tables represents the result obtained on observing the establishment of malaria and typhoid infection by administration of treatmentswith distilled water, different concentration of *Alstoniaboonei* with *Capsicum frutescens*and chloroquine which serves as negative control on parasitized mice to check the effect onplasmodium berghei**(NK 65)/** parasites in established infection(curative), Per Cell Volume,The synergism of *Alstoniaboonei* stem bark and *Capsicum frutescens* extract used exerted significant ($P< 0.05$) dose dependent reduction in percentage parasitemia level at the three (100, 200, and 400)mg/kg doses, While P. *berghe*iparasitaemia was the highest, chloroquine/ciprofloxacin treated mice controlled their infection and presented a significant weight loss compared to the other mice groups, suggesting that weight loss is not a key criterion in this model.The synergism of *Alstoniaboonei* of stem bark with *capsicum frutescens* extracts exhibited a significant curative, suppressive and prophylactic effect against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) in infected mice as demonstrated by the reduction in the level of parasitaemia dose dependently. It is evident based on these findings that Alstoniaboonei possess promising and potent antimalarial and anti-typhoid effect which justifies its usage in folk medicine for the management of malaria.

Keyword; Prophylactic, Curative, Suppressive, Phyto-Medicinal Therapy, *AlstoniaBooneiCapsicumFrutescens*, *Plasmodium Berghei*(NK 65)/*Salmonella Typhi*(ATCC 35723)

1.0 INTRODUCTION

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals and minerals are the basis for treating human diseases (Firenzuoli*et al*.,2007). Medicinal plants are any plant from which valuable drugs can be synthesize as it contains substances that can be used for medicinal purposes (Karim *et al*.,2011). Medicinal plants are presently in demand and their acceptance is increasing progressively. Undoubtedly, plants play an important role by providing essential services in ecosystems. Without plants, humans and other

living organisms cannot live in a way living should be. Anyway, herbals especially medicinal herbs have constantly acted as an overall indicator of ecosystem health (Singh,2002).

Medicinal plants has played a unique holistic role for the provision of food, drugs,clothing,shelter, etc. natural compounds have been extensively explored for new antibiotics drug discoveries (Chavan*et al*.,2017). Indeed, plants have been used as medicines,(Brown *et al*.,2016) as a source of antibiotics, antineoplastic, analgesics, cardioprotective, among others (Chen *et al*.,2015).

In the recent past, humans have been using natural compounds against infections(Newman *et al*.,2016). About 70–90% of the population in developing countries continue to use ancient medicines based on plant extracts and their applications and efficacy (Chin *et al*.,2006). The most powerful and promising elements of plants are their secondary metabolites(Phytochemical), on which humans depend upon (Robinson *et al*.,2011). Significantly, natural products and their derivatives contribute to more than half world antibiotics and antimalariaapproved drugs (Chavan et al.,2018).

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus Plasmodium. The most serious and sometimes fatal type of malaria is caused by Plasmodium falciparum. The other human malaria species, P. vivax, P. ovale, P. malariae, and sometimes P. knowlesi can cause acute, severe illness but mortality rates are low. Malaria is the most important infectious disease in tropical and subtropical regions, and continues to be a major global health problem. It is estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of whom 90% are children in subSaharan Africa [MMV website,2008].

Traditional herbal medicines have been used to treat malaria for thousands of years in various parts of the world. The first antimalarial drug used in the Occident was extracted from thebark of the Cinchona (Rubiaceae) species, the alkaloid quinine, still largely used. Infusions of the plant bark were used to treat human malaria as early as 1632 (Baird *et al.,*1996). Years later quininewas isolated and characterized (Saxena*et al.,*2003), thus becoming the oldest and most important antimalarial drug. Another ancient medicinal plant of millenium use in the West is Artemisia annua, rediscovered in China in the seventies as an important source of the antimalarial artemisinin (Bruce-Chwatt, 1982; Klayman, 1985).

Artemisinin-combined therapies (ACT) were formally adopted asfirst-line treatment of uncomplicated malaria in Nigeria from 2005 onwards (Mokuolu *et al.*,2007). However, ACT use is limited due to its high costs, limited production of artemisinin derivatives to GoodManufacturing Practices (GMP) standards and toxicity (Malomo *et al.*,2001; Borstnik *et al.,* 2002; Adebayo and Malomo, 2002; Afonso *et al.*,2006; Boareto *et al.*,2008).

Alstonia*boonie*, among other plants, has been noted as a good medicinal plant for curing various diseases. Alstonia*.boonei* which belongs to the Apocynaceae family have severally been reported to have medicinal properties. As such, they are used by traditional health practitioners especially in rural areas. Several species of Alstoniabonnie familyincluding Alstonia*macropylla*, Alstonia*scholaris* etc. But this species are not as popular as *Alstoniaboonei,* which have been widely reported in Nigeria. Typically, *Alstoniabooneii*s a facultative plant having estimated occurrence probability of 33 – 67% in both wetland and non-wetland areas. Also *Alstoniabooneii*possesses therapeutic properties probably due to the presence of bioactive constituents and metabolites.

Studies have shown that *Alstoniabooneii*have several medicinal properties both for human and other mammals. For instance studies has indicated that *A. boonei* have Antihyperglycemic (Nkono*et al*., 2014). and antioxidant (Ajaghaku*et al*., 2014), wood healing properties (Mensha*et al*., 2014). Others include Antiplasmodial activities against *Plasmodium bergheiberghei* infection in mice (Osuagwu*et al*., 2015), Analgesic effects (Osuntokun*et al*,.2020), enhancement of rotarod period in albino mice (Okpo*et al*.,2011), diuretic properties in male Wistar rats (Adebayo *et al*.,2014), treatment of chronic Diarrhoea and dysentery, fever, pain, intestinal disorders and as an antidote for Strophanthus poison (Amole *et al*., 2010). anti-snake venom and as antidote to some arrows poisons (Akinloye*et al*., 2013), treatment of malaria, typhoid fever, gonorrhea, yaws, asthma, dysentery, and as a galactagogue (Obogo*et al*.,2013), and antimicrobial properties Different tissues extract of *Alstoniaboonie* have been severally reported to contain some essential secondary metabolite also know as the phytochemicals (Fetse*et al*.,2013).

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Plate 1: Plate showing clockwise order from top right, the leaves, stem, branches of *Alstoniaboonei*

The bark of Alstonia tree is one of the effective analgesic (Abbiw, 1999) herbs available in nature. All the parts of the plant are very useful but the thick bark cut from the matured tree is the part that is most commonly used for therapeutic purposes. The bark of the tree is highly effective when it is used in its fresh form; however, the dried one could equally be used. Therapeutically, the bark has been found to possess ant rheumatic, anti-inflammatory, analgesic/pain-killing, antimalaria/antipyretic, antidiabetic (mild hypoglycaemic), antihelminthic, antimicrobial and antibiotic properties (Hadi and Bremner, 2001). Alstonia decoction also exerts a mild antibacterial effect in this case, relieving the aches and pains associated with malaria fever. Alstonia is taken in the form of preparations that exhibits ant pyrexia and anti-malaria effects, to combat rheumatic and arthritic pains. The decoction of Alstonia bark could be taken alone as an effective pain-killing agent.

Bird pepper- Atawere(Capsicum frutescens) (Cayenne) is an annual or short-lived perennial herb. The stem of Capsicum frutescens almost striatvvmve, glabrous, highier between 1-4 feet depending on climate and growing conditions. The leaves are elliptical, slightly leathery, dark green and smooth, and measure 2½ inches long and 1 inches wide. The flowers are typically conical or funnel form with five petals, usually fused and color is white. The fruits are erect, ellipsoid-conical to lanceoloid, 10-20 mm long, 3-7 mm in diameter. These fruits have range in color from green when immature to purple, red, orange or *Capsicum frutescens* is a species of chili pepper that is sometimes considered to be part of the species *K. Pepper* cultivars of *C. frutescens*can be annual or short-lived perennial plants. Flowers are white with a greenish white or greenish yellow corolla, and are either insect- or self-pollinated. The plants' berries typically grow erect; ellipsoid-conical to lanceoloid shaped. They are usually very small and pungent, growing 10–20 millimeter **Figure 2:** Picture of *Capsicum frutescens*. s (0.39–0.79 in) long and 3–7 millimeters (0.12–0.28 in) in diameter. (Y) Fruit typically grows a pale yellow and matures to a bright red, but can also be othercolors. *Capsicum frutescens* has a smaller variety of shapes compared to other Capsicum species. *Capsicum frutescens*has been bred to produce ornamental strains, because of its large quantities of erect peppers growing in colorful ripening patterns.

Plate 2: *Capsicum frutescens fruit*

Capsicum is a tropical and an important agricultural crop and one of the popular vegetables, not only because of its economic value, but also for the combination of color, taste and nutritional values of its fruit [Kouassi *et al.*,2012;Nadeem *et al.*,2013].

The red colour of mature pepper fruits is due to several related carotenoid pigments, including capsanthin, capsorubin, cryptoxanthin, and zeaxanthin, which are present as fatty acid esters. The most important pigments are capsanthin and its isomer capsorubin, which make up to 30–60% and 6–18% respectively, of the total carotenoids in the fruit [Van *et al.*,2003]. Capsicum frutescens was also used traditionally as an external therapy in painful muscle spasms in areas of shoulder, arm and spine; for treating arthritis, neuralgia, lumbago and chilblains. In addition, it also used for the treatment of diabetes, blood pressure [high/ low], bronchitis, burning feet, to increase circulation, relieve rheumatic pain, treat mouth sores and infected wounds, reduce blood clots, and aid digestion by stimulating saliva and gastric juice flow [Anthony *et al.*,2013;Sunil *et al.*,2012].

2.0 MATTERIAL AND METHOD

2.1; **Plants Sample Collection**:

The medicinal plants stem bark (Alstoniaboonei) and *Capsicum frutescens* fruit were collected from AdekunleAjasin University's reserve forest. The taxonomy of the plants was identified by DrObenben from the Department of Plant Science and Biotechnology (AAUA).

2.2. **Plantsample authentication**:

Both plant samples were authenticated in the herbarium unit of Forest Research Institute of Nigeria (FRIN) with identification number FHI 109806,109872 respectively. They were cleaned to remove sand and other extraneous materials. The medicinal plants used are stem bark of *Alstoniaboonei* and *Capsicum frutescens* respectively

2.3:**Sample preparation and extraction procedure of *Alstoniaboonei* (Stem bark) and *capsicum frutescens*(fruit).**

The stem bark peels were air-dried at room temperature to avoid possible degradation or denaturation of their putative compounds. About 1000g of *Alstoniaboonie* air-dried stem bark and 300g of *Capsicum frutescens* powder was weighed into 500 ml of 80% ethanol and 25% water in cover bottle.

Alstonia.boonei was blended to powder using an electric blender. This was stored in a glass container. Blended air-dried stem bark was soaked in sufficient volume of ethanol for 72 hours at room temperature*C.frutescens* making a percentage of 60:40 percentages. It was continually stirred after each 24 hours. After 72 hours, the mixture was then filtered and the filtrate was concentrated using rotary evaporator at 40^0C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4^0C.(Osuntokun, 2015).

2.4Experimental animals used:

Male Swiss albino mice weighing between 18-20g were obtained from the animal house, Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks in the animal house and fed equally on rat chow and water throughout the period of the experiment.

2.5 Apparatus Used

The apparatus used include beaker, conical for flasks, measuring cylinders, weighing balance, universal centrifuge and volumetric flasks. Thermometer, glass pipette, syringes and needle, test tubes and racks, spatula, glass rod, reagent bottles, water bath, uv-visible spectrophotometer, dissecting board, dissecting set, sample bottles, funnel, oral intubator (cannular), ph meter, microscope, gloves, -20^oc and - 80^oc refrigerator, kidney function and liver function kits, petri-dishes,

2.6 **Parasites**:

The *Plasmodium berghei* was obtained from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan Oyo state, Nigeria. A standard inoculum of 1×10^7 of parasitized erythrocytes from a donor mouse in volumes of 0.2 ml was used to infect the experimental animals intra-peritoneally.

2.7Organism used:

Salmonella typhi used for this experiment was collected from (IAMRAT)) College Of Medicine Ibadan, Oyo State, Nigeria. A standard inoculum of $2x10^8$ acetone- killed *Salmonella typhi*(ty2) with the -ve antigen-free variant 0-901 was used to infect the Swiss Albino mice intra-peritoneally

2.8Test on early malarial / typhoid infection (4-day suppressive test)

The suppressive antimalarialtestalso called"Test on Early Malaria Infection" as reported by Maje 2007 was used in this study. For the Ethanolic and aqueous extracts(separately)Twenty five Swiss albino mice were divided into five groups of five mice each were inoculated with the parasite and organism(*Plasmodium berghei*/*Salmonella typh*) at the commencement of the experiment (day 1). Group's 1-3 mice received 100, 200 and 400mg/kg body weight of extract orally respectively. While the 4th group which served as the positive control received 10mg/kg chloroquine/ Ciprofloxacinbody weight mice in 5th group received 0.2ml distilled water and served as the negative control. On the fifth day (i.e., day 5) the body weight and Packed cell volume were measured and two drops of blood samples from the animal caudal vein(Hoff and Rlatg, 2000;Mohammed et al.,2014) were taken and transferred on slides, thus making thin film from each mouse and staining with Giemsa stain so that average percentage (%) parasitaemia could be evaluated as [Al-Adhroey et al.,2011;Mengiste et al.,2012]

Formulae to calculate the average percentage parasitaemia:

$$\text{Parasitaemia (\%)} = \frac{\text{Total number of PRBC}}{\text{Total number of RBC}} \times 100$$

PRBC = Parasitized red blood cell

RBC = Red blood cell

$$\text{Suppression (\%)} = \frac{\text{Average parasitaemia in negative control --- average parasitaemia}}{\text{Average parasitaemia in negative control}} \times 100$$

2.9:**Test on establishment of malarial and typhoid infection (curative or Rane test)**

Twenty five Swiss albino mice were divided into five groups of five mice each which were inoculated with the parasite and the organism (*Plasmodium berghei*/*Salmonella typhi*) on the first day of the experiment (day 1) The mice were not treated until the parasitaemia was established. On day 4 i.e. 72 hours after the animals were infected. Group's 1-3 mice received 100, 200 and 400mg/kg body weight of the extract per day orally. While the 4th group which served as the

positive control received 10mg/kg chloroquine/Ciprofloxacin orally, mice in 5th group received 0.2ml distilled water and served as negative control for the same period.

On the fifth day (i.e. day 5), the body weight and Packed cell volume were measured and two drops of blood samples from the animals' caudal vein were taken and transferred on slides, thus, making thin film from each mouse and staining with Giemsa stain so that average percentage (%) parasitaemia could be evaluated for each of the doses using the formula above.

After the sixth day, the animal were fed *ad libitum* and observed for 28days. Any death that occurred during this period was noted and used to determine the mean survival time.which can be calculated using the following formula. (Mengiste et al.,2012)

$$\text{Mean Survival Time (MST)} = \frac{\text{sum of survival time(days) of all mice in a group}}{\text{Total number of mice in that group}}$$

2.10**Test on residual malarial and typhoid infection (Prophylaxis test)**

Twenty five Swiss albino mice were divided into five groups of five mice each. Group's 1-3 mice received 100, 200 and 400mg/kg of extract per day orally for three days prior to infection. The 4th group served as positive control and was treated with 10mg/kg body weight of chloroquine/ciprofloxacin while 0.2ml distilled water was given to the fifth group and served as negative control. On the fourth day, standard inoculums of $1x10^7$ *Plasmodium berghei* infected erythrocytes was administered by intrperitoneal route to each mouse. Seventy two hours after, the body weight and packed cell volume were measured and two drops of blood samples from the animals' caudal vein were taken on a slide, thus making a thin film from each mouse and staining with Giemsa stain, examined under the microscope, and percentage chemo suppression determined using the formula above.

2.11:**Statistical Analysis**

The results were expressed in terms of mean ± standard deviation(SD).Parameters in the groups were compared by one-way (ANOVA) using SPSS version 15.level of significance was taken at $p<0.05$.

2.12: **Ethical clearance**

The animals were handled according to the national guidelines for the use and maintenance of experimental animals

3.0: RESULTS

Each of these tables represents the result obtained showing the medicinal efficacy of the medicinal plants against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice, Average body weight, parasite in 4-days suppressive test, Per Cell Volume in 4-day suppressive test, average body weight in 4-days suppressive test, parasite in prophylaxis test, PCV in prophylaxis test and Average body weight in prophylaxis,

1. Curative therapy.

Figure 1 shows Synergistic efficacy of *Alstoniaboonei and Capsicum frutescens* extract against *Plasmodiumberghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino mice (Curative).

Treatment with 10ml/kg of distilled water,the average parasitaemia was 33.11±00.4 and no effect on chemo suppression percentage when injected with 10ml/kg of distilled water. average parasitaemia100mg/kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%) *was* 25.21±0.2and for percentage chemo-suppression was 23.86.Treatment with 200mg/kgof *Alstoniaboonei and Capsicum frutescens*22.12±1.1.and for percentage chemo-suppression was 33.22.average parasitaemia. Treatment with 400mg/kg of *Alstoniaboonei and Capsicum frutescens*was 21.23±0.1,percentage chemo suppression was 35.88.negative control. Treatment with 10mg/kg of chloroquine is 1.443±0.1 and chemo-suppression was measured as 95.68

Table 1: shows Synergistic efficacy of *Alstoniaboonei and Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723)PCV of InfectedSwiss Albino Mice (Curative)**.**

Table 1 shows the changes in per cell volume when treatment with 10ml/kg of distilled water,per cell volume was 28.1±0.2 .Treatment with 100mg /kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)was *32.3*±0.1. Treatment with 200mg/kg of *Alstoniaboonei* and*Capsicum frutescens*was 33.3±0.2. Treatment with 400mg/kg of *Alstoniaboonei*and *Capsicum frutescens was* 34.4 ±0.4, and treatment with 10mg/kg chloroquine which serves as negative control ,the PCV measured was as 36.4±1.1.

Table 2: shows Synergistic efficacy of *Alstoniaboonei*and*Capsicum frutescens* extract against *Plasmodiumberghei*(NK 65)/*Salmonella typhi*(ATCC 35723)Average body weight of InfectedSwiss Albino Mice (Curative)**.**

Table 2 shows the changes in average body weight when treated with 10ml/kg of distilled water. the average body weight measured was 16.3± 0.2,Treated with 100mg/kg of*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%),the average body weight was 18.0±0.1.Treatment with 200mg/kg of *Alstoniaboonei*and *Capsicum frutescens* was 18.7±1.1. Treatment with 400mg/kg of *Alstoniaboonei*and *Capsicum frutescens*, there was a change in average body weight, 19.2±0.2. Treatment with chloroquine as negative control, the average body weight measured as 21.3 ± 0.1**.**

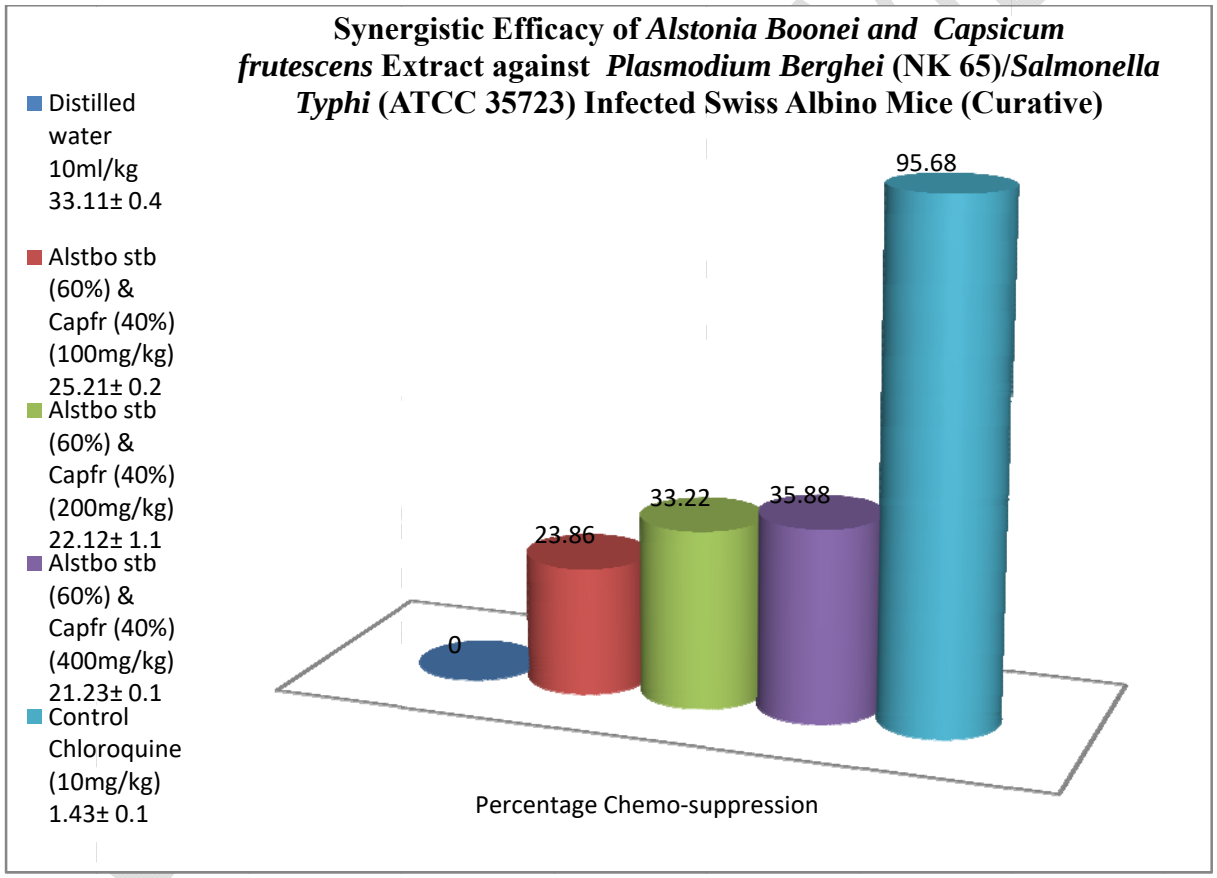

Figure **1: Synergistic Efficacy of *Alstoniaboonei*and*Capsicum frutescens* extract against *Plasmodiumberghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice (Curative and Percentage chemo-suppression)**

Table 1: Synergistic Efficacy of *Alstoniaboonei*and*Capsicum frutescens* extract against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723)PCV of InfectedSwiss Albino Mice (Curative)

TREATMENTS	PCV (%)
Distilled water 10ml/kg	28.1±0.2
***Alstbos*tb (60%)&*Capfr*(40%)**(100mg/kg)	32.3±0.1
***Alstbos*tb (60%)&*Capfr*(40%)** (200mg/kg)	33.3±0.2
***Alstbos*tb (60%)&*Capfr*(40%)** (400mg/kg)	34.4±0.1
Chloroquine (10mg/kg)	36.4±1.1

Key- Extract -***Alstoniaboonies*tem bark (60%) and *Capsicum frutescens*(40%)**

Table 2: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723),Average body weight of InfectedSwiss Albino Mice

TREATMENTS	Weight (g)
Distilled water 10ml/kg	16.3 ± 0.2
***Alstbos*tb (60%)&*Capfr*(40%)** (100mg/kg)	18.0 ±0.1
***Alstbos*tb (60%)&*Capfr*(40%)** (200mg/kg)	18.7 ±1.1
***Alstbos*tb (60%)&*Capfr*(40%)** (400mg/kg)	19.2 ± 0.2
Chloroquine (10mg/kg)	21.3 ± 0.1

2. Suppressive therapy

Table 3: :Synergistic efficacy of *Alstoniaboonei*and*Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test).

Table 3 shows the Average parasitaemiaof*plasmodium berghei*and *Salmonella typhi* parasite in 4-day suppressive test. Treatment with 10mg/kg of distilled water,29.16± 0.4 and chemo-suppression was zero.The average parasitaemiatreatmentofplasmodium b*erghe*i parasite in 4-day suppressive test with100mg/kg*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)yielded 20.70± 1.2 and the Percentage Chemo-suppression was29.01. The Average parasitaemiatreatmentofplasmodium berghei parasite in 4-day suppressive test with200mg/kg*Alstoniaboonei*and *Capsicumfrutescens*yielded 15.96± 0.3 and the Percentage Chemo-suppression was45.27. TheAverage parasitaemiatreatmentof*plasmodium berghei* parasite in 4-day suppressive test with400mg/kg*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)yielded 12.13± 1.1and the Percentage Chemo-suppression was 58.40.Treatment with chloroquine as negative control, the average parasitaemia was 3.56± 0.2 and the chemo-suppression was 87.79

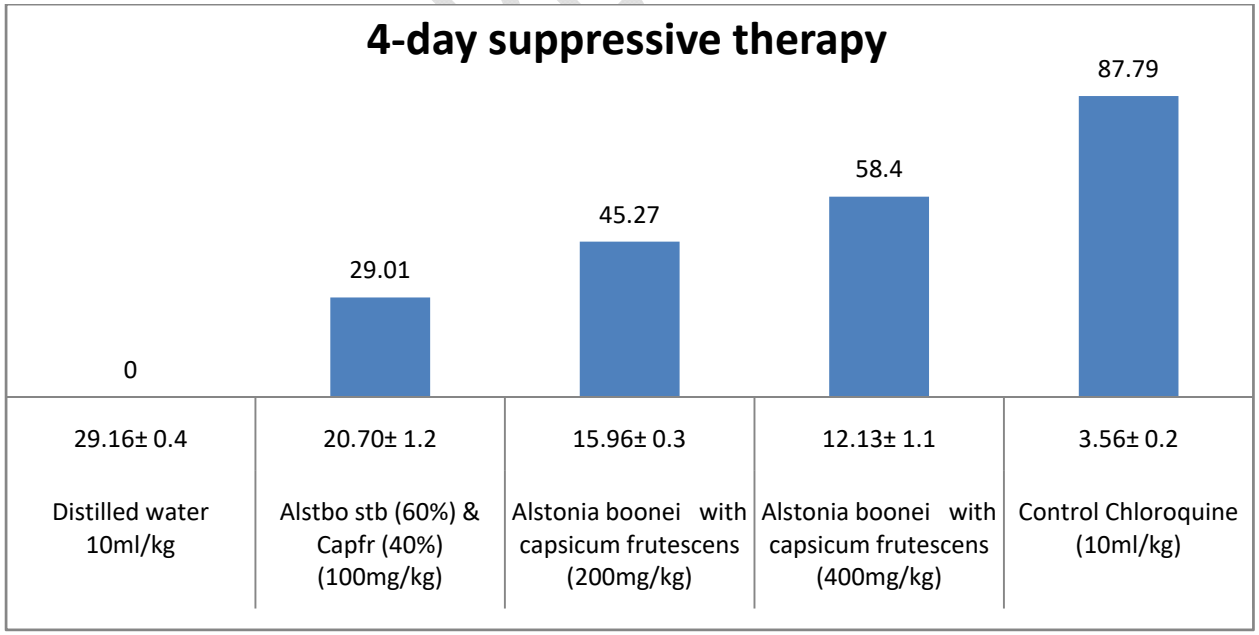

Figure 2: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test and Percentage Chemo-suppression).

Table 4: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test).Per Cell Volume (PCV).

The effect of 10ml/kg of distilled water on per cell volume in 4-day suppressive test was 33.1±0.1.change in PCV in 4-day suppressive test when treated with 100ml/kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)was 37.3±1.4.Treatment with 200ml/kg of *Alstoniaboonei* and *Capsicum frutescens*, per cell volume was39.3±0.4.change in PCV in 4-day suppressive test.Treatment with 400ml/kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)was 39.4±0.3 and the change in PCV in 4-day suppressive test. Treatment with 10mg/kg of chloroquine was 40.9±1.1

Table 4: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test).Per Cell Volume (PCV).

TREATMENTS	**PCV (%)**
Distilled water 10ml/kg	33.1±0.1
***Alstbo*stb (60%)&*Capfr*(40%)** (100mg/kg)	37.3±1.4
***Alstbo*stb (60%)&*Capfr*(40%)** (200mg/kg)	39.3±0.4
(400mg/kg)	39.4±0.3
Chloroquine (10mg/kg)	40.9±1.1

Key- Extract -***Alstoniaboonie*stem bark (60%) and *Capsicum frutescens*(40%)**

Table 5: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test).Average body weight in 4-day suppressive test

Table 5 shows the effect of treatments on average body weight in 4-day suppressive test when treated with 10mg/kg of distilled water which yielded 17.2 ± 0.1. Treatment with 100mg/kg of*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%),the average body weight in 4-day suppressive test was 19.2 ±0.3. Treatment with 200mg/kg of *Alstoniaboonei* and *Capsicum frutescens*,the average body weight in 4-day suppressive test was 20.3 ±1.3. Treatment with 400mg/kg of*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%),the average body weight in 4-day suppressive test was 21.7 ± 0.1. when treated with chloroquine as the negative control, average body weight in 4-day suppressive test it yielded 22.1 ± 1.1.

Table 5: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test).Average body weight in 4-day suppressive test

TREATMENTS	**Weight (g)**
Distilled water 10ml/kg	17.2 ± 0.1
Alstoniaboonei with *capsicum frutescens* (100mg/kg)	19.2 ±0.3
Alstoniaboonei with *capsicum frutescens* (200mg/kg)	20.3 ±1.3
Alstoniaboonei with *capsicum frutescens* (400mg/kg)	21.7 ± 0.1
Chloroquine (10mg/kg)	22.1 ± 1.1

Key- Extract -***Alstoniaboonie*stem bark (60%) and *Capsicum frutescens*(40%)**

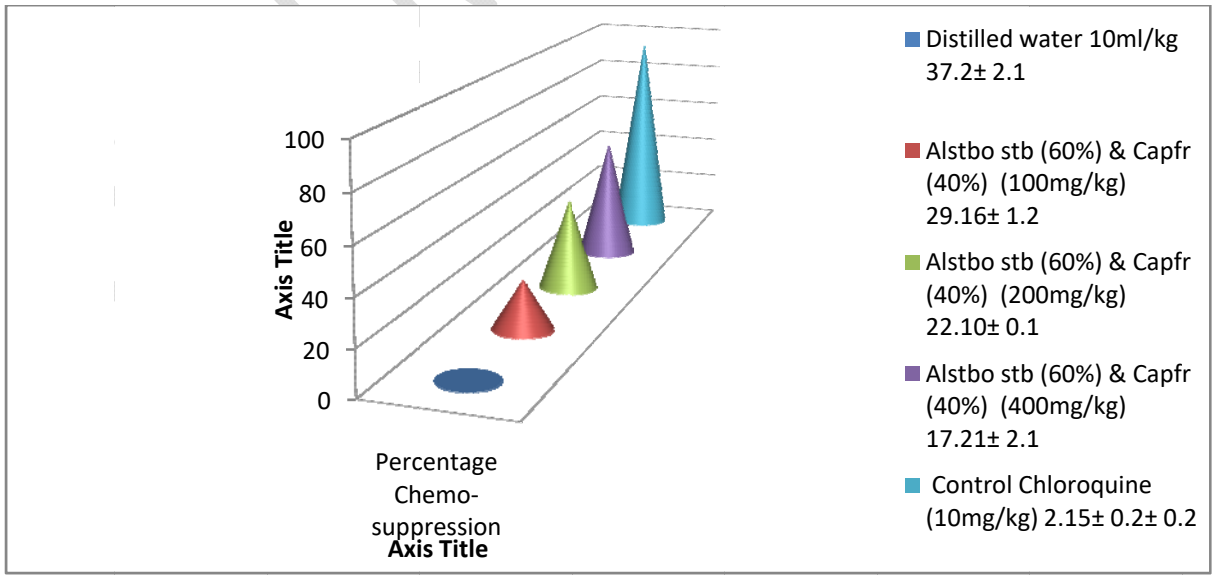

Figure 3: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice(4-day suppressive test and percentage chemo suppression).Average body weight.

3.Prophylaxis therapy

Table 6: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice (PCV, Prophylaxis test)

The effect of 10ml/kg of distilled water per cell volume in Prophylaxistest was 32.1± 1.2. There was a change in PCV in Prophylaxis test when treated with 100mg/kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%)was 36.1±1.1. PCV in Prophylaxis test when treated with 200ml/kg of *Alstoniaboonei* and *Capsicum frutescens*, per cell volume was38.2 ± 1.2,PCV in Prophylaxis test when treated with 200ml/kg of *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%), per cell volume was 39.2±0.3 and PCV in PCV in Prophylaxis test when treated with 10mg/kg of chloroquine was 40.2± 1.2

Table 6: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice (PCV, Prophylaxis test)

TREATMENTS	PCV (%)
Distilled water 10ml/kg	32.1± 1.2
***Alstbos*tb (60%)*&Capfr*(40%)** (100mg/kg)	36.1±1.1
***Alstbos*tb (60%)*&Capfr*(40%)** (200mg/kg)	38.2±1.2
***Alstbos*tb (60%)*&Capfr*(40%)** (400mg/kg)	39.2±0.3
Chloroquine (10mg/kg)	40.2± 1.2

Key- Extract -***Alstoniaboonie*stem bark (60%) and *Capsicum frutescens*(40%)**

Table 7: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice (Average body weight, Prophylaxis test)

Table 7 shows the change in average body weight when treated with 10mg/kg of distilled which yielded 15.5±0.1 body weight. Treatment with100mg/kg of*Alstoniaboonei*(60%) *and Capsicum frutescens*(40%), the average body weight measured was 19.1±2.0. Treated with 200mg/kg *Alstoniaboonei* and *Capsicum frutescens*, the average body weight measured was 24.3±1.1. Treatment with 400mg/kg *Alstoniaboonei*(60%) *and Capsicum frutescens*(40%), the average body weight measured was 24.3±1.1.Treatment with 10mg/kg chloroquinne which serves as the negative control, average body weight in prophylaxis test was 24.4±1.2.

Table 7: Synergistic efficacy of *Alstoniaboonei*and *Capsicum frutescens* extract against Plasmodium*berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) InfectedSwiss Albino Mice (Average body weight, Prophylaxis test)

TREATMENTS	Weight(g)
Distilled water 10ml/kg	15.5±0.1
***Alstbos*tb (60%)&*Capfr*(40%)** (100mg/kg)	19.1±2.0
***Alstbos*tb (60%)&*Capfr*(40%)** (200mg/kg)	24.3±1.1
***Alstbos*tb (60%)&*Capfr*(40%)** (400mg/kg)	24.1±0.2
Chloroquine (10mg/kg)	24.4±1.2

Key- Extract -***Alstoniaboonies*tem bark (60%) and *Capsicum frutescens*(40%)**

4.0 DISCUSSION

The purpose of this research work is to evaluates the potential medicinal activity of ***Alstoniaboonei and*** *Capsicum frutescens* extracts for Prophylactic, Curative & Suppressive phytomedicinal therapy against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723)against malaria and typhoid using swiss albino mice.

The problem of antibiotic resistance has posed a debilitated challenge on the choice of antimalaria /anti-typhoid drugs. To face this critical public health problem and improve the management of malaria and typhoid cases, home-based management is the most implemented measure [Aubouy,2011; Ferrer *et al.*,2016], but the use of traditional medicine in a safe, cost-efficient and effective manner also constitutes a way to ensure that all people have access to health care. However, for in vivo validation of safety and efficiency to be achieved; The present study aimed to evaluate prophylaxis, suppressive, curative and anti-malarial,anti-typhoidal efficacy of crude extract of *Alstoniaboonei* and*capsicum frutescens in* synergism, to improve the use and drug formulation of herbal based drug,conventional and synthetic drug.

In the 4-day suppressive test performed in the P. berghei model, weight loss was lower in Alstoniaboonei and *Capsicum frutescens*treated mice compared to those that received chloroquine/ciprofloxacin. This finding cumulates the fact that medicinal plants in use may increasing effect of this crude extract on food intake or appetite. The synergism of *Alstoniaboonei* stem bark and *Capsicum frutescens* extract used exerted significant ($P< 0.05$) dose dependent reduction in percentage parasitemia level at the three (100, 200, and 400)mg/kg doses, While P. *berghe*iparasitaemia was the highest, chloroquine/ciprofloxacin treated mice controlled their infection and presented a significant weight loss compared to the other mice groups, suggesting that weight loss is not a key criterion in this model. In the most severe malaria model with P. *berghei,* administration of *Alstoniaboonei*and *Capsicum frutescens*in mice induced similar weight changes compared to chloroquine/ciprofloxacin, strengthening the safety of *Alstoniaboonei* with Capsicum *frutescens* at the tested doses. Weight loss differences were observed between treatments at Day 5 when all untreated mice have died(Osuntokun*et al*., 2020).

The Antiplasmodial activity of *Alstoniaboonei* and*Capsicum frutescens* extracts was confirmed in vivo in the *P. berghei* model. *Alstoniaboonei*and Capsicum *frutescens* -treated mice displayed about 40% chemosuppression when the peak of infection was reached in untreated mice. This significant result reflects an inhibitory activity on parasite replication in this model. This activity was verifiedin the *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723)synergistic model.

However, significant increased survival of *P. berghei* infected mice treated with plant extracts and chloroquine/ciprofloxacin was obtained in comparison with untreated mice.At day 5 all untreated mice died whereas 66.7 and 50% of mice that received *Alstoniaboonei* and *Capsicum frutescens* survived, respectively. This result indicates a very interesting ability to extend survival in this model characterized by a high mortality rate. However, survival to P. *berghei/Salmonella typhi*infected mice was related to parasite elimination in *Alstoniaboonei* and *capsicum frutescens*treated mice. In this model, death is due to neuroinflammation related to influx of myeloid immune cells to the brain, oxidative stress, blood brain barrier permeability and neurodegeneration [Isah*et al.*,2014, Howland *et al*.,2015].The synergistic ethanolic extracts of *AlstoniaBoonei*with *Capsicum frutescens*exhibited comparable suppressive activity on P. *berghe/Salmonella typhi* which is in agreement with previous work on in vitro antimalarial activities (Ali *et al.,* 2002).

From the present study, synergistic activity of*Alstoniaboonei*and *Capsicum frutescens* plants extracts exhibited promising suppressive activity on P.*berghei*/*Salmonella typhi*. The highest suppression in both plant extracts was shown at the maximum dose given (400 mg/kg). This might be due to the fact that the active compounds ,responsible for the antimalarialactivity ,mostly occur in low levels in natural products and activity may not be detected in lower doses (Krettl*et al.,* 2009).Studies have also shown the efficacy of alkaloids and flavonoids in plants (Balogun,2009). The observable features of antimalarial/anti-typhoid potential in the extract treated group may be attributed to the presence of various secondary metabolites (Phytochemical). The PCV values obtained in the result showed an improvement over therapeutic treatment.

. CONCLUSION

The synergism of *Alstoniaboonei* of stem bark with *capsicum frutescens* extracts exhibited a significant curative, suppressive and prophylactic effect against *Plasmodium berghei*(NK 65)/*Salmonella typhi*(ATCC 35723) in infected mice as demonstrated by the reduction in the level of parasitaemia dose dependently. It is evident based on these findings that Alstoniaboonei possess promising and potent antimalarial and anti-typhoid effect which justifies its usage in folk medicine for the management of malaria.

Recommendation

In view of these findings, efforts should be made to further:

i. Characterize the active components of this plant

ii. Elucidate the mechanisms of action of its components on malaria parasite.

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