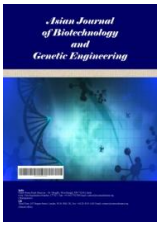

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Curative, Suppressive and Prophylactic Phyto-Medicinal Therapy/Synergistic Efficacy of *Alstonia boonei*/*Capsicum frutescens* Ethanolic Extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice

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Authors' contributions

Author OTO researchs into different arrays of medicinal plants and helps to designed the materials and methods, proof-read the first and final manuscript for constrictive criticism. Author ITF helps manage interprets and analyzed the collected data for statistical analysis. Both authors read and approved the final manuscript

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ABSTRACT

The purpose of this research work is to evaluates the medicinal activity of *Alstonia boonei and Capsicum frutescens* extracts for Prophylactic, Curative & Suppressive phytomedicinal therapy against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice**.** *Alstonia boonei* belongs to the Apocynaceae family and has several medicinal properties, for the treatment of various diseases while *Capsicum frutescens* is a tropical plant, an important agricultural crop and a popular African vegetables. The stem bark of *Alstonia boonei* and fruit of *Capsicum frutescens* were collected from Adekunle Ajasin 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. *Plasmodium berghei* (NK 65) and *Salmonella Typhi*

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(ATCC 35723) from the Institute of Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Nigeria. The animals were acclimatized for two weeks and fed equally on rat chow and water throughout the period of the research work. 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 antimalarial test also called"Test on Early Malaria Infection" was used in this study. Twenty five Male Swiss albino mice weighing between 18-20g were obtained from the animal house, the experimental animal 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). 72 hours. Group's 1-3 mice received 100, 200 and 400mg/kg body weight of the extract per day orally. While the 4th group served as the positive control received 10mg/kg Chloroquine/Ciprofloxacin orally, 5th group received 0.2ml distilled water and served as negative control for the same period. Result obtained on observed the establishment of malaria and typhoid infection by administration of treatments dosage with different concentration of *Alstonia boonei* and *Capsicum frutescens*. The synergism of *Alstonia boonei* stem bark and *Capsicum frutescens* extract with significant ($P< 0.05$) dose dependent reduction in percentage parasitemia level at the three (100, 200, and 400) mg/kg doses, While P. *berghe*i parasitaemia was the highest, Chloroquine /Ciprofloxacin, a significant weight loss compared to the other mice groups, suggesting that weight loss is a key criterion in this model. The synergism of *Alstonia boonei* of stem bark and *Capsicum frutescens friut* extracts exhibits a significant curative, suppressive and prophylactic potency 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 *Alstonia boonei / Capsicum frutescens* possess promising and potent antimalarial and anti-typhoid potential which justifies its usage in folk medicine for the management of malaria and typhoid infection.

Keywords: Alstonia boonei; curative; Capsicum frutescent; Plasmodium berghei (NK65); phyto-medicinal therapy; prophylactic; suppressive; Salmonella typhi (ATCC 35723); Mean Survival Time (MST).

1. INTRODUCTION

Natural products from plants and animals are the basis for treatment of human diseases. Medicinal plants are any plant from which valuable drugs can be synthesize as it contains substances that can be used for medicinal purposes [1]. Medicinal plants are presently in high 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, herbals especially medicinal herbs have constantly acted as an overall indicator of ecosystem health [2]. Medicinal plants have played a unique holistic role for the provision of food and drugs. Natural compounds have been extensively explored for new antibiotics drug discoveries [3]. Indeed, plants have been used as medicines [4] as a source of antibiotics, antineoplastic, analgesics, cardioprotective, among others [5]. In the recent past, humans have been using natural compounds against infections [6]. About 70–90% of the population in developing countries continues to use ancient medicines based on plant extracts and their applications and efficacy [7]. The most powerful and promising elements of plants are their secondary metabolites (Phyto chemical), on which humans depend upon [8]. Significantly, natural products and their derivatives contributes to more than half world antibiotics and antimalaria approved drugs [3].

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*, *P. knowlesi* can causes acute, severe illness but mortality rates are very low. Malaria is the most important infectious disease in tropical and subtropical regions (Africa), 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 sub Saharan Africa [9].

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 the

stem bark of the Cinchona (Rubiaceae) species, the alkaloid quinine, still largely used. Infusions of the plant stem bark were used to treat human malaria. Years later quinine was isolated and characterized [10], 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 [11].

Artemisinin-combined therapies (ACT) were formally adopted as first-line treatment of uncomplicated malaria in Nigeria from 2005 onwards [12]. However, ACT use is limited due to its high costs, limited production of artemisinin derivatives to Good Manufacturing Practices (GMP) standards and toxicity [13-17].

Typhoid fever, a potentially life-threatening gastrointestinal infection, which is caused by a non-spore bearing bacilli called *Salmonella Enterica* serovar typhi (*S.typhi*)[18]. This bacterium is transmitted by faecal-oral route with the organism gaining entry into the body through the intestinal mucosa [19]. Advances in public health strategies, technology, and hygiene have led to the eradication of typhoid fever from the developed world, but since the 1800s, typhoid fever has remained an endemic disease in many developing countries [20], because of the high morbidity and mortality associated with typhoid fever [21], particularly in children under 5 years old [22,20].

The sources of infection vary but the most common mode of transmission is by ingesting an infective dose of *S. typhi* through contaminated food or water. The true global disease burden of typhoid fever is difficult to be estimated as few established surveillance systems on typhoid fever exist in developing countries [20].In Africa, the overall burden of typhoid fever remains largely unknown, mainly because facilities capable of performing the blood culture tests essential for diagnosis are absent from many regions [23].

Alstonia boonie, among other plants, has been noted as a good medicinal plant for the treatment of 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 *Alstonia bonnie* family including *Alstonia macropylla, Alstonia scholaris* etc. this species are not as popular as *Alstonia boonei,* which have been widely reported in Nigeria. Typically, *Alstonia boonei* is a facultative plant having estimated occurrence probability of 33 – 67% in both wetland and non-wetland areas. Also *Alstonia boonei* possesses therapeutic properties probably due to the presence of bioactive constituents and metabolites [23].

Alstonia boonei have several medicinal properties both for human and other mammals. For instance studies have been indicated that *A. boonei* have Antihyperglycemic [24]. Antioxidant [25], Wound healing properties [26]. Others include Antiplasmodial activities against *Plasmodium berghei* infection in mice [27], Analgesic effects [28], Enhancement of rotarod period in albino mice [29], Diuretic properties in male Wistar rats [30], Treatment of chronic Diarrhea and dysentery, fever, Pain, Intestinal disorders and as an antidote for Strophanthus poison [31]. Anti-snake venom and as antidote to some arrows poisons [32]. Treatment of malaria, Typhoid fever, Gonorrhea, Yaws, Asthma, Dysentery, and as a galactagogue and antimicrobial properties Different tissues extract of *Alstonia boonie* have been severally reported to contain some essential secondary metabolite also known as the phytochemicals [33].

The stem bark of *Alstonia boonei* tree is one of the effective analgesic [34] herbs available in nature. All the parts of the plant are very useful but the thick stem bark cut from the matured tree is the part that is most commonly used for therapeutic purposes. The stem 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 Stem bark has been found to possess Anti-rheumatic, Anti-inflammatory, Analgesic/pain-killing, Anti-malaria /Antipyretic, Anti-diabetic (Mild hypo glycaemic), Anti helminthic, Antimicrobial and Antibiotic properties [35]. *Alstonia boonei* decoction also exerts a mild antibacterial effect in this case, relieving the aches and pains associated with malaria fever. *Alstonia boonei* is taken in the form of preparations that exhibits antipyrexia and anti-malaria effects, to combat rheumatic and arthritic pains. The decoction of *Alstonia boonei stem* bark could be taken alone as an effective pain-killing agent.

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. Bird

pepper- Ata-were in Yoruba language ,Nigeria ,West Africa. *Capsicum frutescens* (Cayenne) is an annual or short-lived perennial herb. The stem bark of *Capsicum frutescens* almost 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. 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 [28].

Plate 1. Plate showing clockwise order from top right, the leaves, stem, branches of *Alstonia boonei*
Source: Osuntokun et al, 2017[36]

Plate 2. *Capsicum frutescens fruit*
Source (Osuntokun et al,. 2020(28)

Plate 2, Picture of *Capsicum frutescens*.(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 other colors. *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.

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 [37,38]. 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 [39]. *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 [40,41].

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

The medicinal plants stem bark of *Alstonia boonei* and fruit of *Capsicum frutescens* were collected from Adekunle Ajasin University's reserve forest. The taxonomy of the plants was identified by Dr Obenben from the Department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko. Ondo state,Nigeria.

2.2. Plant Samples 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 *Alstonia boonei* and *Capsicum frutescens* respectively.

2.3 Samples Preparation and Extraction Procedure of *Alstonia boonei (*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 *Alstonia boonie* 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 24 hours. After 72 hours, the mixture was then filtered and the filtrate was concentrated using rotary evaporator at 40^{o}C. The concentrate was heated over a water bath to obtain a solvent free extract, which was stored in a refrigerator at 4^{o}C [42].

2.4a. Experimental Animals Used For Experimental Analysis

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. The experimental animals were group into the following group as follows on experimental design.

2.4b. Experimental Design

Group: 1 Distilled water (10ml/kg)

Group: 2 Synergistic ethanol extract of *Alstonia boonei* (60%) *and Capsicum frutescens* (40%) (100 mg/kg).

Group: 3 Synergistic ethanol extract of *Alstonia boonei*(60%) *and Capsicum frutescens* (40%) (200 mg/kg).

Group: 4 Synergistic ethanol extract of *Alstonia boonei*(60%) *and Capsicum frutescens* (40%) (400 mg/kg).

Group: 5 Control (Chloroquine /Ciprofloxacin)

2.5 Apparatus used for Experimental Analysis

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^{o}c and - 80^{o}c refrigerator, kidney function and liver function kits, petri-dishes.

2.6 Parasites used for Experimental Analysis

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 inoculums 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-peritoneal.

2.7 Organism used for Experimental Analysis

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

2.8 Test on Early Malarial/Typhoid Infection (4-Day Suppressive Test)

The suppressive antimalarial test also called "Test on Early Malaria Infection" as reported by Majekodunmi, 2007 [43] was used in this study. For the Ethanol and aqueous extracts (sepa rately)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 400 mg/kg body weight of extract orally respectively. While the 4th group which served as the positive control received 10mg/kg Chloroquine/Cipro floxacin body 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 [44,45] 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 [46,47]

Formulae to calculate the average percentage parasitaemia:

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

PRBC = Parasitized red blood cell
RBC = Red blood cell

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

2.9 Test on Establishment of Malarial/ 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 28 days. 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 [48].

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

2.10 Test on Residual Malarial/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 10 mg/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 inter-peritoneal 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 [48].

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 $p<0.05$.

3. RESULTS

Each of these tables represents the results obtained showing the medicinal efficacy of the medicinal plants against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice, Average body weight, parasite in 4-days suppressive test, Packed-cell volume in 4-day suppressive test, average body weight in 4-days suppressive test, parasite in prophylaxis test, Parked cell volume (PCV) in prophylaxis test and Average body weight in prophylaxis,

Fig. 1 shows Synergistic efficacy of *Alstonia boonei/Capsicum frutescens* extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss 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 10 ml/kg of distilled water. average parasitaemia 100mg/kg of *Alstonia boonei* (60%) *and Capsicum frutescens* (40%) *was* 25.21±0.2 and for percentage chemo-suppression was 23.86.Treatment with 200mg/kg of *Alstonia boonei and Capsicum frutescens* 22.12±1.1.and for percentage chemo-supp ression was 33.22. average parasitaemia. Treatment with 400mg/kg of *Alstonia boonei and Capsicum frutescens* was 21.23±0.1, percentage chemo suppression was 35.88.negative control. Treatment with 10mg/kg of Chloroquine/ Ciprofloxacin was 1.443±0.1 and chemo-supp ression was measured as 95.68.

Fig. 2 shows the Average parasitaemia of *plasmodium berghei* and *Salmonella typhi* parasite in 4-day suppressive test. Treatment with 10 mg/kg of distilled water, 29.16± 0.4 and chemo-suppression was zero. The average parasitaemia treatment of *plasmodium berghei* parasite in 4-day suppressive test with 100 mg/kg *Alstonia boonei* (60%) *and Capsicum frutescens* (40%) yielded 20.70± 1.2 and the Percentage Chemo-suppression was 29.01. The Average parasitaemia treatment of *Plasmodium berghei* parasite in 4-day suppressive test with 200mg/kg *Alstonia boonei* and *Capsicum frutescens* yielded 15.96± 0.3 and the Percentage Chemo-suppression was 45.27. The Average parasitaemia treatment of *Plasmodium berghei* parasite in 4-day suppressive test with 400mg/kg *Alstonia boonei* (60%) *and Capsicum frutescens* (40%) yielded 12.13± 1.1 and the Percentage Chemo-suppression was 58.40. Treatment with Chloroquine/Ciprofloxacin as negative control, the average parasitaemia was 3.56± 0.2 and the chemo- suppression was 87.79.

Fig. 3 shows the Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (4-day Prophylactic test and percentage chemo suppression).Average body weight. The plant extract shows a promising activity next to the synthetic drug chloroquine/ Ciprofloxacin.

3.1 Curative Therapy (Tables 1 & 2)

Table 1 shows the changes in per cell volume when treatment with 10 ml/kg of distilled water, per cell volume was 28.1±0.2.Treatment with

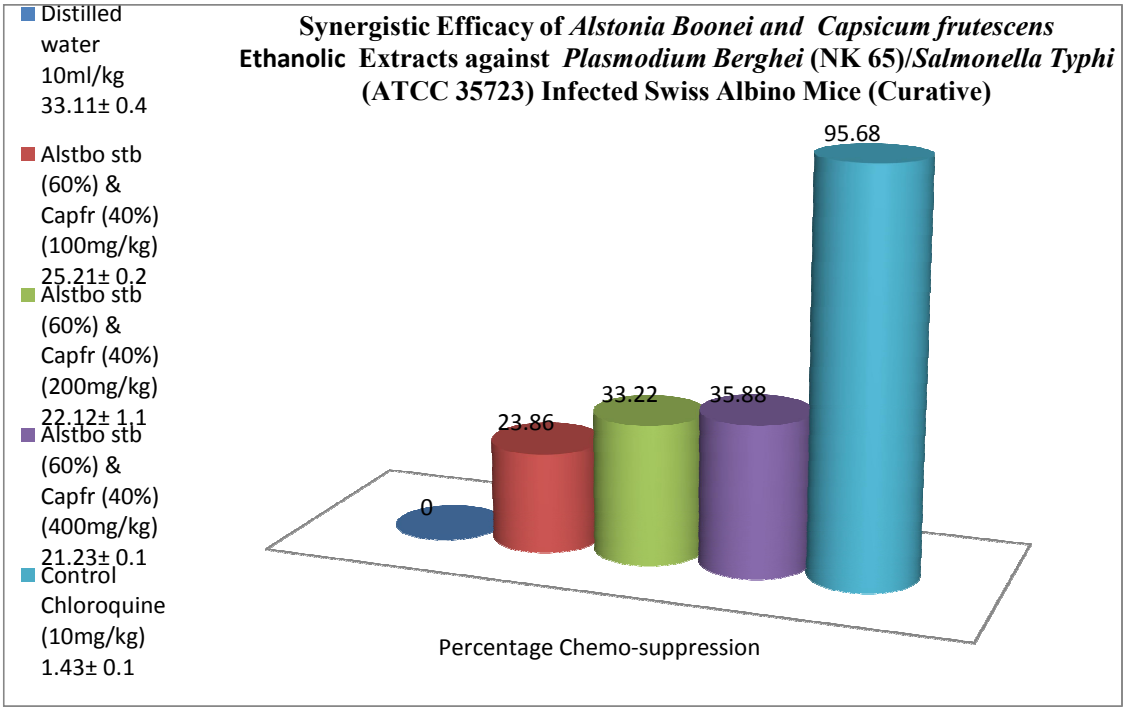

Fig. 1. Synergistic Efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (Curative and Percentage chemo-suppression)

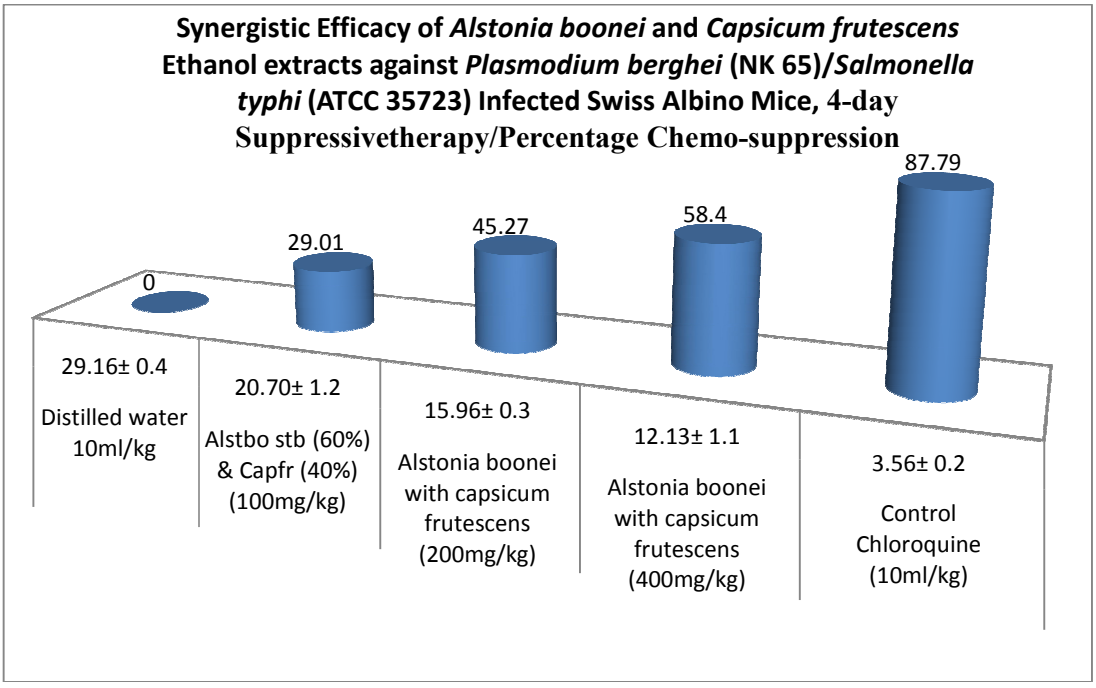

Fig. 2. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (4-day suppressive test and Percentage Chemo-suppression)

Fig. 3. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against Plasmodium *berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice(4-day Prophylactic test and percentage chemo suppression).Average body weight

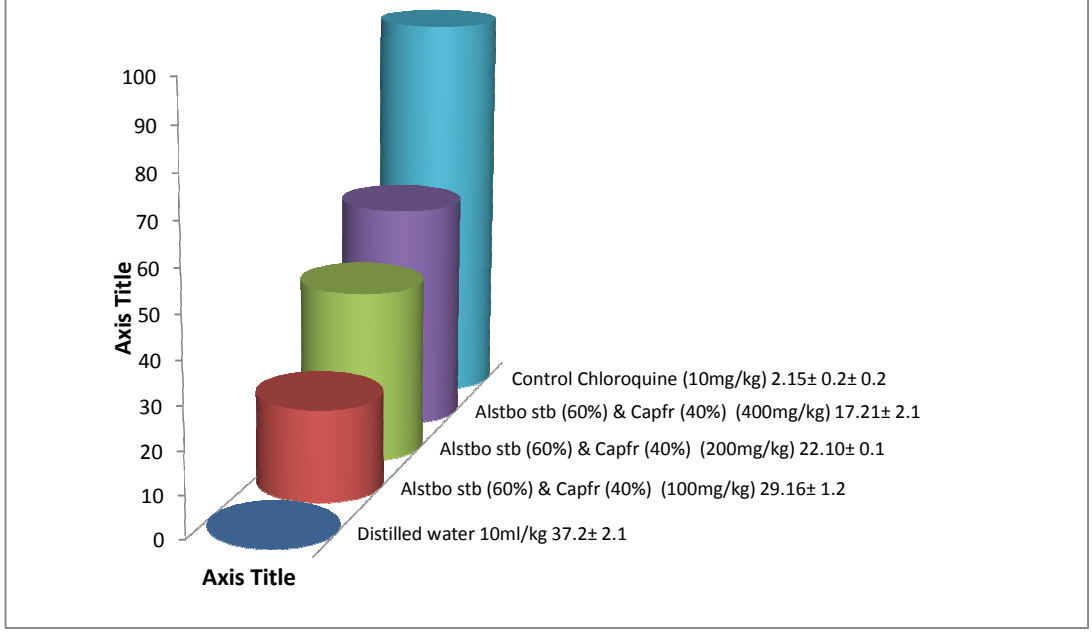

Fig. 3. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice(4-day Prophylactic test and percentage chemo suppression).Average body weight

Fig. 4. Percentage suppression of ethanolic extract *Alstonia boonie* stem bark (60%) and *Capsicum frutescens*(40%) on early malaria/typhoid infection in Swiss Albino Mice (%)

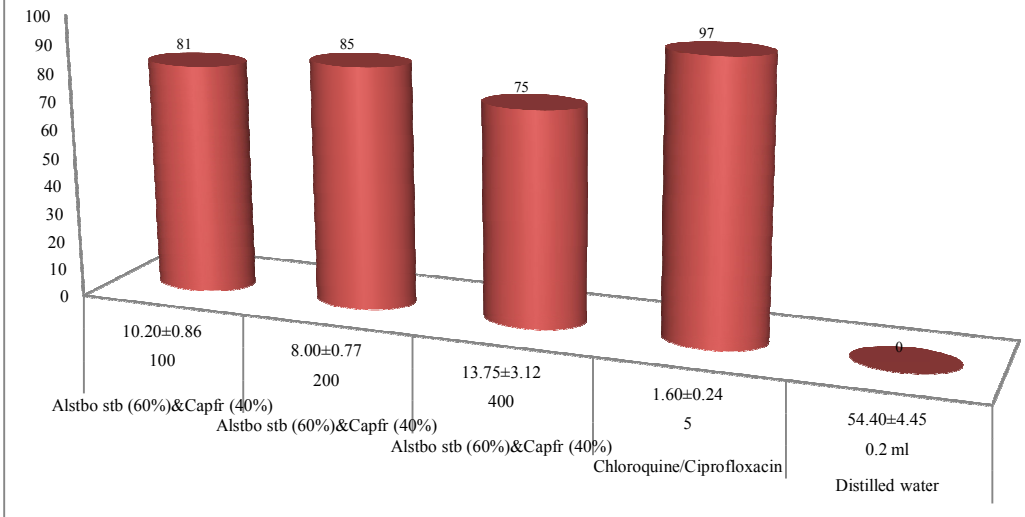

Fig. 4. Percentage suppression of ethanolic extract *Alstonia boonie* stem bark (60%) and *Capsicum frutescens*(40%) extracts on early malaria/typhoid infection in Swiss Albino Mice (%)

Table 1. Synergistic Efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Packed-cell volume (PCV) of Infected Swiss Albino Mice (Curative)

Treatments	Packed-cell volume (PCV) (%)
Distilled water 10 ml/kg	28.1±0.2
Alstbo stb (60%) & *Capfr* (40%) (100 mg/kg)	32.3±0.1
Alstbo stb (60%) & *Capfr* (40%) (200 mg/kg)	33.3±0.2
Alstbo stb (60%) & *Capfr* (40%) (400 mg/kg)	34.4±0.1
Chloroquine / Ciprofloxacin (10 mg/kg)	36.4±1.1

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens(40%), Mean ± SE

Table 2. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723), Average body weight of Infected Swiss Albino Mice

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

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens(40%) Mean ± SE

Table 3. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (4-day suppressive test). Packed-cell volume (PCV)

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

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens(40%) Mean ± SE

100 mg/kg of *Alstonia boonei* (60%) *and Capsicum frutescens* (40%) was *32.3*±0.1. Treatment with 200 mg/kg of *Alstonia boonei* and *Capsicum frutescens* was 33.3±0.2. Treatment with 400 mg/kg of *Alstonia boonei* and *Capsicum frutescens was* 34.4 ±0.4, and treatment with 10 mg/kg Chloroquine/Ciprofloxacin which serves as negative control, the Packed-cell volume (PCV) measured was as 36.4±1.1.

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

3.2 Suppressive Therapy (Tables 3 & 4)

The effect of 10ml/kg of distilled water on per cell volume in 4-day suppressive test was 33.1±0.1.change in Packed-cell volume (PCV) in 4-day suppressive test when treated with 100 ml/kg of *Alstonia boonei* (60%) *and Capsicum frutescens extracts* (40%) was 37.3±1.4.Treat ment with 200 ml/kg of *Alstonia boonei* and *Capsicum frutescens extracts*, Packed-cell volume was 39.3±0.4. change in PCV in 4-day suppressive test. Treatment with 400 ml/kg of *Alstonia boonei* (60%) *and Capsicum frutescens extracts* (40%) was 39.4±0.3 and the change in Packed-cell volume (PCV) in 4-day suppressive test. Treatment with 10 mg/kg of Chloroquine/ Ciprofloxacin was 40.9±1.1.

Table 4 shows the effect of treatments on average body weight in 4-day suppressive test

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

3.3 Prophylaxis Therapy (Tables 5 & 6)

The effect of 10ml/kg of distilled water per cell volume in Prophylaxis test was 32.1± 1.2. There was a change in Packed-cell volume (PCV) in Prophylaxis test when treated with 100 mg/kg of *Alstonia boonei* (60%) *and Capsicum frutescens extracts* (40%) was 36.1±1.1. Packed-cell volume (PCV) in Prophylaxis test when treated with 200 ml/kg of *Alstonia boonei* and *Capsicum frutescens*, per cell volume was 38.2 ± 1.2, PCV in Prophylaxis test when treated with 200ml/kg of *Alstonia boonei* (60%) *and Capsicum frutescens extracts* (40%), Packed-cell volume was 39.2± 0.3 and PCV in PCV in Prophylaxis test when treated with 10mg/kg of Chloroquine/ Ciprofloxa cin was 40.2± 1.2.

Table 6 shows the change in average body weight when treated with 10 mg/kg of distilled which yielded 15.5±0.1 body weight. Treatment with100mg/kg of *Alstonia boonei* (60%) *and Capsicum frutescens extracts* (40%), the average body weight measured was 19.1±2.0. Treated with 200 mg/kg *Alstonia boonei* and *Capsicum frutescens*, the average body weight

Table 4. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (4-day suppressive test).Average body weight in 4-day suppressive test

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

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens(40%) Mean ± SE

Table 5. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (Packed-cell volume (PCV), Prophylaxis test)

Treatments	Packed-cell volume PCV (%)
Distilled water 10 ml/kg	32.1± 1.2
Alstbo stb (60%) & *Capfr* (40%) (100 mg/kg)	36.1±1.1
Alstbo stb (60%) & *Capfr* (40%) (200 mg/kg)	38.2±1.2
Alstbo stb (60%) & *Capfr* (40%) (400 mg/kg)	39.2±0.3
Chloroquine/ Ciprofloxacin (10 mg/kg)	40.2± 1.2

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens(40%) Mean ± SE

Table 6. Synergistic efficacy of *Alstonia boonei* and *Capsicum frutescens* Ethanol extracts against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) Infected Swiss Albino Mice (Average body weight, Prophylaxis test)

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

Key- Extract -Alstonia boonie stem bark (60%) and Capsicum frutescens (40%) Mean ± SE

measured was 24.3±1.1. Treatment with 400 mg/kg *Alstonia boonei* (60%) *and Capsicum frutescens extracts*(40%), the average body weight measured was 24.3±1.1.Treatment with 10 mg/kg Chloroquinne/ Ciprofloxacin which serves as the negative control,average body weight in prophylaxis test was 24.4±1.2.

4. DISCUSSION

The purpose of this research work is to evaluates the potential medicinal importance of *Alstonia boonei and Capsicum frutescens* Ethanol extracts. The present study aimed to evaluate prophylaxis, suppressive, curative and anti-malarial/anti-typhoidal efficacy of crude extracts of *Alstonia boonei* and *capsicum frutescens in* synergism, against *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) against malaria and typhoid using Swiss albino mice, to improve the use and drug formulation of herbal based drug, conventional and synthetic drug.

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 and natural phytomedicinal therapy is the most implemented measure and therapy of choice [49,50], 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 proper medical health care at low cost especially for the third world country.

P. berghei/Salmonella typhi model, weight loss was lower in *Alstonia boonei* and *Capsicum frutescens extract* treated mice compared to those that received Chloroquine/Ciprofloxacin. This finding cumulates the fact that medicinal plants in use may increase food intake or appetite. In the ethanolic extracts of *Alstonia boonei and Capsicum frutescens extracts,* it was observed that at 100, 200, and 400mg/kg, there was a moderate increase in the activity of the plant extract compare to the synthetic drug Chloroquine/Ciprofloxacin with the curative, 4-day suppressive test and Prophylactic test [51]. This suggests that the pharmacological active ingredients of these plants responsible for its antimalarial/antityphoid activities may be localized in the extract as their secondary metabolites. Chloroquine/Ciproflaxin treatment group showed highest chemo-suppression in the infected mice based on the fact that the parasite is sensitive to Chloroquine / Ciprofloxacin and it has been purified compared with the crude extracts of *Alstonia boonei and Capsicum frutescens* that were used [52].

This current study shows an increment chemo-suppressive, curative and prophylactics potency with the use of ethanol solvent than the previous reports with the use of methanol as a result of solvent used by the present study being a good extractive solvent and plant environment has been proven to contribute to the chemo therapeutic efficacy [52].

The synergism of *Alstonia boonei* stem bark and *Capsicum frutescens* extract used has significant ($P< 0.05$) dose dependent reduction in curative, 4-day suppressive test and Prophylactic test at 100, 200, and 400mg/kg doses, The prophylactic and curative model showed no significant ($p \geq 0.05$) loss of body weight except groups treated with 200 mg/kg for prophylactic assay. The loss of body weight in this group might be due to decrease in appetite and reduced suppressive ability of the crude extract on infected mice since it was given before infection [53].

The safety of *Alstonia boonei* with *Capsicum frutescens extracts* at the tested doses were observed between day 4 and 5. There was a significant weight loss and increased death rate(Mortality),this signify, that between day 4 and 5, the extract becomes toxic to the mice, due to the increase in toxic compound which is shown in the internal organs of the mice [54].

The Antiplasmodial activity of *Alstonia boonei* and *Capsicum frutescens* extracts were confirmed in vivo in the *P. berghei/Salmonella typhi* model. *Alstonia boonei* and *Capsicum frutescens extract* -treated mice displayed about 40% chemosuppression at peak of infection at day 3,4,5 and also there is an increase in the bio-activity of plant at day 3,4 and 5. This significant result reflects an inhibitory activity on parasite replication in this model. This activity was verified in the *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) synergistic model [36].

However, significant increased survival of *Plasmodium berghei* (NK 65)/*Salmonella typhi* (ATCC 35723) 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 *Alstonia boonei* and *Capsicum frutescens extract* 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 medicinal plant phytotherapy of reduction in the rate of parasite potency in *Alstonia boonei* and *capsicum frutescens* treated mice [53].

In this model of medicinal plant phytotherapy, death was due to neuro-inflammation related to influx of myeloid immune cells to the brain, oxidative stress, blood/ brain barrier permeability and neuro-degeneration (54).The synergistic ethanol extracts of *Alstonia boonei* with *Capsicum frutescens extracts* exhibited comparable suppressive activity on P*. berghe/Salmonella typhi* which is in agreement with previous work on in vitro antimalarial activities [55].

From the present study, synergistic activity of *Alstonia boonei* 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 antimalarial /antityphoid activity ,mostly occur in moderate dosage in natural products and activity may not be detected in lower doses [56] and studies have also shown the efficacy of alkaloids and flavonoids in plants [57,58] in related to the bioactive compound found in medicinal plants and observable features of antimalarial/anti-typhoid potential of *Alstonia boonei and Capsicum frutescens* Ethanol extracts treated group may be attributed to the presence of various secondary metabolites (Phytochemical), this shows the PCV values obtained in the result showed an improvement over therapeutic treatment because of the presence of secondary metabolites [59].

5. CONCLUSION

The synergism of stem bark of *Alstonia boonei* and fruit of *Capsicum frutescens* extracts exhibited a significant Curative, Suppressive and Prophylactic efficacy 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 *Alstonia boonei* and *Capsicum frutescens* has a promising and potent anti malarial/anti-typhoid activities which justifies its usage in folk medicine, for the management of malaria/tyhoid fever over the conventional synthetic drug. its usage must be encouraged.

6. RECOMMENDATION

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

- Characterize the active components of this plant
- Elucidate the mechanisms of action of its components on malaria parasite.

ETHICAL APPROVAL

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

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Karim A, Sohail MN, Munir S, Sattar S. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int. J. Pharmacol. 2011;7:419-439.
2. Singh G, Maurya S, de Lampasona MP, Catalan CAN. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem. Toxicol. 2007;45:1650-166.
3. Chavan SS, Damale MG, Devanand B. Antibacterial and Antifungal Drugs from Natural Source: A Review of Clinical Development; Natural Products in Clinical Trials: Sharjah, UAE. 2018;1:114
4. Brown ED. Wright GD. Antibacterial drug discovery in the resistance era. Nature. 2016;529:336–343.

5. Chen S, Song J, Sun C, Xu J, Zhu Y, Verpoorte R, Fan TP. Herbal genomics: Examining the biology of traditional medicines. Science. 2015;347:27–S29
6. Newman DJ. Natural products as leads to potential drugs: an old process or the new hope for drug discovery? Journal Medical Chemistry. 2008;51:2589–2599.
7. Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. AAPS J. 2006;8:E239–E253.
8. Robinson MM, Zhang X. The World Medicines Situation 2011, Traditional Medicines: Global Situation, Issues and Challenges; World Health Organization: Geneva, Switzerland, 2011;1-4.
9. Klayman DL. Qinghaosu (artemisinin): an antimalarial drug from China Science. 1985;228:1049-1055
10. Saxena SN, Pant DC, Jain RS. Bhakuni Antimalarial agents from plant sources Current Science. View Record in Scopus Google Scholar. 2003;85:1314-1329
11. Bruce-Chwatt Qinghaosu LJ. A new antimalarial British Medical Journal (Clinical Research Ed.), Cross Ref View Record in Scopus Google Scholar. 1982;284:767-768.
12. Mokuolu OA, Okoro EO, Ayetoro SO, Adewara AA. Effect of artemisinin-based treatment policy on consumption pattern of antimalarials American Journal of Tropical Medicine and Hygiene. 2007;76:7-11.
13. Malomo SO, Adebayo JO, Olorunniji FJ. Decrease in activities of cation ATPases and alkaline phosphatate in kidney and liver of artemether treatead rats NISEB Journal. (2001);1:175-182.
14. Borstnik K, Paik IH, Shapiro TA, Posner GH.Antimalarial chemotherapeutic peroxid es: artemisinin, yingzhaosu A and related compounds International Journal of Parasitology. 2002;32:1661-1667
15. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review.JournalEthnopharmacology.2011;1 33: 289-302
16. Afonso A, Hunt P, Cheesman S, Alves AC, Cunha CV, doRosario V, Cravo P. Malaria parasites can develop stable resistance to artemisinin but lack mutations in candidate genes atp6 (encoding the sarcoplasmic and endoplasmic reticulum Ca^{2+} ATPase), tctp, mdr1, and cg10 Antimicrobial Agents Chemotherapy. 2006;50;480-489
17. Boareto AC, Muller JC, Bufalo AC, Botelho GGK, deAraujo SL, Foglio MA, de Morais RN, Dalsenter PR. Toxicity of Artemisinin [Artemisia annua L.] in two different periods of pregnancy in Wistar rats Reproductive Toxicology. 2008;25:239-246.
18. Santos RL, Zhang S, Tsolis RM, Kingsley R, Adams GL, Baumler A. Animal models of Salmonella infections: Enteritis versus typhoid fever.Microbes Infect. 2001;3 (14) :1335-1344.DOI:10.1016/j.etp.2011.01.01 4.
19. Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, Bhutta ZA, Canh DG, Ali M, Shin S, Wain J, Page AL, Albert MJ, Farrar J, Abu-Elyazeed R, Pang T, Galindo CM, von Seidlin L, Clemens JD. Domi typhoid study group. A study of typhoid fever in fiveAsian countries: Disease burden and implications for controls. Bull. World Health Organ. 2008;86:260-268.
20. Bhutta ZA, Dewraj HL. Current concepts in the diagnosis and treatment of typhoid fever.BMJ. 2006;333(7558):78-82.
21. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents).Braz J Med Biol Res. 2000; 33(2): 179-89.
22. Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, Bhutta ZA. Typhoid fever in children: Some epidemiological considerations. Int. J. Infect. Dis. 2006;10:215-222.
23. Mweu E, English M. Typhoid fever in children in Africa.Trop. Med. Int. Health. 2008;13(4):532-540.
24. Nkono RLNY, Sokeng SD, Désiré DDP, Kamtchouing P. Antihyperglycemic and Antioxydant Properties of *Alstonia boonei* De Wild. (Apocynaceae) Stem Bark Aqueous Extract in Dexamethasone-Induced Hyperglycemic Rats, International Journal of Diabetes Research. 2014;3 (3):27-35.
25. Ajanaku KO, Ajanaku CO, Edoboh-Osoh A, Nwinyi OC. Nutritive value of sorghum ogi fortified with groundnut seed. The American Journal of Food Technology. 2012;7(2):82–88.Available:https://doi. org/10.3923/ajft.2012.82.88
26. Mensah AY, Donkor PO, Fleischer TC. Anti-Inflammatory and Antioxidant Activi ties Of The Leaves Of Wissadula Amplissima Var Rostrata. African Journal of Traditional, Complementary and Alter native Medicines. 2011;8.

27. Osuagwu GGE, Onwuegbuchulam NP. The Phytochemical Screening and Antimicrobial Activity of the Leaves of Monodora myristica, (Gaertn) Dunal, Acanthus montanus (Ness) T. Anders and Alstonia bonnie De Wild, International Journal of Pharmacy and Pharmaceutical Research. 2015;2(4):85 – 102.
28. Oludare Temitope Osuntokun, Pius John Ajiga. Alstonia boonie & *Capsicum frutescens* extract on *Plasmodium berghei* (Nk 65) / Salmonella typhi (ATCC 35723) Infected Swiss Albino Mice, International Journal of Research and Innovation in Applied Science (IJRI AS) |. 2020;5(3) |ISS N:2454-6194:1-13Available:www.Rsis inter national.org
29. Stephen O Okpo, Fidelis P Ching, Buniyamin A Ayinde, Onajite O Udi, Peter O Alonge, Gerald O Eze. Gastroprotective Effects of the Ethyl Acetate Fraction of Anchomanes difformis (Engl) International Journal of Health Research. 2011;4(4) :155-161
30. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: A review. Journal Ethnopharmacology. 2011 ;133: 289-302.
31. Amole OO, Ilori OO. Antimicrobial activity of the aqueous and ethanolic extracts of the stem bark of *Alstonia boonei*. Int. J. Phytopharmacol. 2010;1(2):119–123.
32. Akinloye OA, Oshilaja RT, Okelanfa OA, Akinloye DI, Idowu O. Hypoglyceamic Activity of *Alstonia boonei* Stem Bark Extract in Mice, Agriculture and Biology Journal of North America. 2013;4(1):1-5.
33. Fetse JP, Kyekyeku JO, Dueve E, Mensah KB. Wound Healing Activity of Total Alkaloidal Extract of the Root Bark of *Alstonia boonei* (Apocynacea), British Journal of Pharmaceutical Research. 2014;4(23):2642-265.
34. Abbiw D.Useful Plants of Ghana: West African Uses of Wild and Cultivated Plants Intermediate Technology Publications, Royal Botanical Garden, Kew, London. 1990;2(4);390-396.
35. Hadi V, Bremner JB. "Initial studies on alkaloids from Lombok medicinal plants," Molecules. 2001;6(2):117– 129.
36. Oludare Temitope Osuntokun, Bamidele O, Aladejana OM, Ogunlade AO. Evaluation of Antimicrobial Activity, Synergistic Efficacy, Qualitative and Quantitative Phytochemical Determination of *Alstonia boonei* Leaf and Stem Bark on Selected Clinical Isolates, Triple A Research Journal of Multidisciplinary (JMD). 2017;1(1):001- 014.
37. Kouassi KC, Koffi NR. Evaluation de la connaissance et utilisation des varietes de piment (Capsicum) cultivees en Cote d'Ivoire. International Journal of Biological and Chemical Sciences. 2012;6 (1):75-85
38. Nadeem M, Anjum FM, Khan MR, Saeed M and Riaz M. Antioxidant potential of bell pepper (Capsicum annuum L.)-A review. Pak J Food Sci. 2011;21(1-4):45-51.
39. Van RS, Bosaini E, Mayr D, Pugh G, Posthumus M. Evaluation of three gas chromatography and two direct mass spectrometry techniques for aroma analysis of dried red bell peppers .International Journal of Mass Spectrome try. 2003;22355-65.
40. Anthony OE, Ese AC, Lawrence EO. Regulated effects of *Capsicum frutescens* supplemented diet (C.F.S.D) on fasting blood glucose level, biochemical parameters and body weight in alloxan induced diabetic Wistar rats. British Journal of Pharmaceutical Research. 2013;3(3):496-507.
41. Sunil C, Somashekar RK, Nagaraja BC. Riparian vegetation dynamics across two different landscapes along the river Cauvery in the Kodagu region of western ghats. Journal of Mountain Science. 2012;9:351-361
42. Osuntokun OT, Oluwafoise BO. Phytochemical Screening of Ten Nigerian Medicinal Plants, International Journal of Multidisplinary Research and Development . 2015;2(4):390-396.
43. Majekodunmi SO. "Formulation of the extract of the stem bark of *Alstonia boonei*as tablet dosage form," Tropical Journal of Pharmaceutical Research. 2007;7(2):987–994,
44. Hoff J, Rlagt LV. Methods of blood collection in the mouse. Lab animals. 2000;29:47–53.
45. Muhammad NA. Amusa. The important food crops and medicinal plants of North-western Nigeria Research Journal of Agricultural and Biological Science. (2005);1:254-260.
46. Al-Adhroey AH, Nor ZM, Al Mekhlafi HM, Amran AA, Mahmud R. Evalu ation of the use of Cocos nucifera as antimalarial remedy in Malaysian folk medicine J. Ethnopharmacol. 2011;134(3):988-991, DOI:10.1016/j.jep.2011.01.026

47. Mengiste E, Makonnen, Urga K. “In vivo antimalarial activity of Dodonaea Angust ifolia seed extracts against Plas modium berghei in mice model,” Momona Ethiopian Journal of Science. 2012;1:47–63.
48. Ogu GI, Tanimowo WO, Nwachukwu PU, et al. Antimicrobial and phytochemicals of root extracts of Cyathuta prostate (L) Blume against some pathogens. J Intercult Ethnopharmacol. 2012;1(1):35-43.
49. Aubouy A, Bakary M, Keundjian A, Mbomat B, Makita JR, Migot-Nabias F, Cot M, Le Bras J, Deloron P. Combination of drug level measurement and parasite genotyping data for improved assessment of amodiaquine and sulfadoxine-pyrim ethamine efficacies in treating Plasmodium falciparum malaria in Gabonese children. Antimicrob Agents Chemother. 2003; 47: 231-237.DOI:10.1128 /AAC.47.1.231-237.2003.
50. Ferrer A, Remon S, Negueruela AI, Oria R. Changes during the ripening of the very late season Spanish peach cultivar Calanda: Feasibility of using CIELAB coordinates as maturity indices. Scientia Hort. 2005;105:435-446.
51. Bantie LM. In vivo antimalarial activity of the crude root and fruit extracts of Croton macrostachyus (Euphorbiaceae) against *Plasmodium berghei* in mice. Journal of Traditional and Complementary Medicine. 2014;4:1–6.DOI:10.4103/22254110.124323.
52. Oladunmoye MK, Kehinde FY. Ethno botanical survey of medical plants used in treating viral infections among Yoruba tribe of South Western Nigeria. African. Journal of Microbiology Research. 2011;5 (19) :2991–3004.
53. Ebiloma GU, Omale J, Aminu RO. Suppressive, currative and prophylactic potentials of Morinda lucida (Benth) against erythrocytic Stage of mice infective chloroquine sensitive *Plasmodium berghei* NK-65. British Journal of Applied Science and Technology. 2011;1(3):131- 140.
54. Howland SW, Poh CM, Rénia L. Activated brain endothelial cells cross-present malaria antigen. PLoS Pathog. ;11:e1004 963; 2015.
55. Isah MB, Ibrahim MA. The role of antioxidants treatment on the pathogenesis of malarial infections: a review. Parasitol Res. 2014;113:801–9.
56. Alli LA, Adesokan AA, Salawu OA, Akanji MA, Tijani AY. Antiplasmodial activity of aqueous root extract of Acacianilotica.Africa Journal. Biochemistry Research. 2011;5(7):214-219ISSN 1996-0778.\
57. Krettli AU, Adebayo JO, Krettli LG. Testing of natural products and synthetic molecules aiming atnewanti malarials. Current Drug Targets. 2009;10;261–270.
58. Noland GS, Graves PM, Sallau A, Eigege A, Emukah E, Patterson AE. Malaria prevalence, anaemia and baseline intervention coverage prior to mass net distribution in Abia and Plateau States, Nigeria. BMC Infection Diseases. 2014;14 :166–172.
59. Abiodun OM, Balogun OR. Sexual Activity and Contraception Use among Young Female Students of Tertiary Institutions in Ilorin, Nigeria. Contraception. 2009;79:146-149.Available:http://dx.doi.org/10.1016/j. contraception.2008.08.00

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