IN-VIVO ANTI-PLASMODIAL ACTIVITY OF ETHANOL *TETRACARPIDIUM CONOPHORUM* SEED EXTRACT AND ITS ANTI-TYPHOIDAL ACTIVITY *IN-VITRO*

ABSTRACT

Aim: This study was conducted to assess the *in-vivo* anti-plasmodial activity of ethanol *Tetracarpidium conophorum* seed extract and its anti-typhoidal activity *in-vitro.*
Study Design: Experimental study.
Methodology: Standard methods were conducted to determine the acute toxicity test of *Tetracarpidium conophorum* seed ethanol extract, determination of Body Weight and Temperature of Mice, determination of antibacterial activity of *Tetracarpidium conophorum* seed crude extract and antibiotic sensitivity testing of clinical and typed *Salmonella ~~Typhi~~ typhi* isolates
Results: There were no signs of toxicity such as paw licking, sleeping, reduced activity, respiratory distress observed in mice and there were no mortality. Changes occurred in the weight of mice in group 1 (mice treated with 200mg/kg) from 19.71g to 14.50g wherein significant increment was observed only from day 3 to 4 (18.67g to 16.00g), group 2 (mice treated with 400mg/kg) 19.71g to 16.00g). The ethanol extract of *Tetracapidium conophorum* were all resistant to typed isolates of *Salmonella typhimurium* and clinical isolates of *Salmonella typhimurium* at 6.01±0.10 mm. Ciprofloxacin (5 µg) had the highest zone of inhibition at 32.50±2.50 mm against typed *Salmonella typhimurium* while tetracycline (30 µg) had the least at 15.50±0.50 mm.
Conclusion: This study has revealed the anti-plasmodial efficacy of *Tetracarpidium conophorum* seed. Findings have shown that the ethanol seed extract of *T. conophorum* relatively possess anti-plasmodial and anti-typhodial activities ~~compared to~~as well as the positive antibiotic susceptibility of *Salmonella typhi*. Auxiliary evaluation must be conducted to establish the anti-typhoidal activity of ethanol *T. conophorum* extract *in-vivo*

INTRODUCTION

Malaria has been a severe and life threatening disease for thousands of years. The major impact of the disease is entirely on the developing countries, with the heaviest burden in Africa. It is one of the leading infectious diseases in many tropical regions, including Nigeria [1]. According to Bloland, [2], malaria is a complex disease whose epidemiology and clinical manifestations varies widely in different parts of the world as a result of the species of malaria parasite, their susceptibility to anti-malarial drugs, distribution and efficiency of mosquito vectors, climate and level of immunity of the exposed human population. This parasitic disease is transmitted by the bite of an infected female *Anopheles* mosquito. Five *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae* and *Plasmodium ovale*) that infect human were recognized [3]. *Plasmodium falciparum* is the most dominant, pathogenic and said to be responsible for almost all mortality caused by malaria in tropical and Sub tropical countries where the temperature and rainfall are optimum for the development of vectors and parasites [5]. *Plasmodium berghei* is transmitted by the bite of the disease vector, mosquito ~~(~~ species known as *Anopheles dureni~~)~~.* These parasites of rodents are practical model organisms in the laboratory for the study of human malaria aimed at the development of new vaccines and treatments [6].

On African walnut (*Tetracarpidium conophorum*), Ihemeje *et al*. (2015) stated that it a tropical ram-bling perennial woody plant of the family Euphorbiaceae which is widely distributed and consumed by the inhabitants of Africa. The plant is often found growing wild as a climber in the forest regions of Africa and India. Edem *et al*, (2017) reported that *T.conophorum* has a long history as food plant and is grown by peasant farmers. The plant is mostly cultivated for its nuts which can be cooked and consumed as snacks. In Nigeria, it is "Asala or Awusa" in Yoruba, "Ukpa" in Igbo and "Okhue or Okwe" in Edo. *T. conophorum*, like many plants in Africa and other parts of the world has been proven to have decorative, nutritive, medicinal, agricultural and industrial values over the years. *Tetracarpidium conophorum* (family Euphorbiaceae) seed is known as African walnut. In Nigeria, it is 'Ukpa' in Igbo and 'Awusa 'or Asala in Yoruba [10]. It is a west equatorial perennial plant often found growing in forests distributed in the southern part of Nigeria and West Africa. *Tetracarpidium conophorum* seed is highly medicinal and has been reported to be effective in the treatment of malaria [11]. The seeds are rich in flavonoid, tannins, alkaloid, protein, carbohydrate, fat and oils, vitamins and minerals which enhance its antimicrobial activities [12].

According to Budd (2012), *Salmonella enterica* subspecies *enterica* serovar Typhi (*Salmonella typhi*) is the cause of typhoid fever. Together, *Salmonella typhi* and *Salmonella* serovar Paratyphi A are the major agents of enteric fever. Like other typhoidal *Salmonella* serovars, *Salmonella typhi* is a human host–restricted organism. Soper, (2017) said that the role of water and food has been a vehicle to typhoid fever whic has been appreciated since the late 1800s. WHO, (2017), noted that understanding of the global burden of typhoid fever has improved in recent decades, with an increase in both the number and geographic representation of high-quality typhoid fever incidence studies and greater sophistication of modeling approaches. According to Sood *et al.* (2014), the emergence of multidrug resistance among the enteric fever group of *Salmonella* to the first line antibiotics such as ampicillin, chloramphenicol and cotrimoxazole has been a major concern.

Hasan *et al.* (2018) stated that in countries with a higher incidence of multi drug resistance (MDR) isolates, *S. paratyphi* displays a higher level of resistance towards fluoroquinolones compared to *S.* Typhi. Ochiai *et al.* (2018) explained that the problem only worsened with the advent of Nalidixic acid resistant *Salmonella typhi* (NARST) making ciprofloxacin a doubtful drug of choice for the treatment of enteric fever with the changing patterns in antibiogram it is necessary to continually monitor the drug resistance pattern and understand the mechanisms involved. Hence the rationale for this study is to assess the *in-vivo* anti-plasmodial activity of ethanol *Tetracarpidium conophorum* seed extract and its anti-typhoidal activity *in-vitro.*

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MATERIALS AND METHODS

Collection and grouping of experimental Mice

Albino mice used in this study were treated in conformity with International National and Institutional guidelines for care and use of laboratory animals for Biomedical Research by Canadian council of Animal Care and United State National Institute of Health described by (Ogundolie *et al*., 2017). A total of 35 mice was randomly divided into five groups in which seven mice are in a group. Group 1 was treated with 200mg/ml of the extract while group 2 was treated with 400mg/ml of the extract and group 3 given 800mg/ml of the extract. Group 4 was treated with chloroquine and group 5 was the negative control fed with water for the anti-malarial activity and 15 mice was randomly grouped into three groups of five mice per group for acute toxicity test. The mice were given 200mg/ml of the extract to group 1,400mg/ml of the extract to group 2 and 800mg/ml of the extract to group 3 for the acute toxicity test.

Acute toxicity test of *Tetracarpidium conophorum* seed ethanol extract

The acute toxicity test of *Tetracarpidium conophorum* was carried out using modified Lorkes (1983) method as described by Ogundolie *et al*. (2017). The extract was prepared by dissolving 2.0g, 3.0g, 4.5g and 5.0g into 5ml volumes of distilled water separately to produce concentrations of 200mg/ml, 400mg/ml and 800mg/ml respectively which was then administered orally to each mouse in the groups.

Collection of Parasites

Chloroquine-sensitive strain of malaria parasite (*Plasmodium berghei* NK 65) in a donor mouse was obtained from Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The injection of the mice with *Plasmodium berghei* NK 65 and observation for behavioral changes including; decreased activities, loss of appetite was conducted as described by Yerbanga *et al*. (2016).

Determination of Body Weight and Temperature of Mice

According to (Dada and Muhammed, (2018), the body weight of each mouse in all groups was measured before and after acute toxicity test at different doses, using sensitive digital weighing balance (Weight milk Water). Temperatures of mice were measured using a thermometer which was inserted into the rectum. The mice body weight and temperature were read during the four days of experiment.

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Source and Preservation of *Salmonella typhi*

Pure clinical and typed isolates of *Salmonella typhi* were obtained from the stock culture gotten from the Department of Microbiology of the Federal University of Technology Akure, Ondo State. It was prepared into slant before it was used.

Preparation of bacterial inoculums

According to Dada and Faleye (2016), the bacterial inoculum three isolated overnight colonies were transferred to a tube of sterile saline. The bacterial suspension was compared to the 0.5 McFarland standards against a sheet of white paper on which sharp black lines were drawn. The bacterial suspension was adjusted to be the proper density as the 0.5 McFarland by adding sterile saline or more bacterial growth.

Determination of antibacterial activity of *Tetracarpidium conophorum* seed crude extract

Method described by Clinical and Laboratory Standards Institute (CLSI, 2017) was used for the antimicrobial assay of extracts of the plant was performed by agar well diffusion method using Mueller Hinton Agar (MHA) (Oxoid Basingstokes, UK). The test bacterium was inoculated in Nutrient broth and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml. MHA plate was lawn cultured with standardized microbial culture broth. Plant extracts of 50 mg/ml concentration were prepared in Dimethyl Sulfoxide (DMSO). Four wells of 6 mm were bored in the inoculated media with the help of sterile cork-borer (6 mm). Each well was filled with 50 μl extracts of the plants: positive control (amikacin 30 µg and nitrofurantoin 300 µg) for bacteria and negative/solvent control (DMSO), respectively. It was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37 °C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm using a vernier caliper.

Antibiotic sensitivity testing of clinical and typed *Salmonella Typhi* isolates

Antibiotic susceptibility tests were performed on the isolates using standard agar diffusion techniques as described by Clinical Laboratory Standard Institute (CLSI, 2017) and commercially available antibiotics. All susceptibility tests were carried out using overnight cultures. The isolates were subcultured from slants onto freshly prepared nutrient agar plates and incubated at 35 °C for 18 h. They were further sub-cultured into Mueller-Hinton broth and incubated for 6h. A 1ml suspension of each bacteria isolate, equivalent to McFarland standards was aseptically seeded into Mueller Hinton agar plates respectively. This was allowed to stand for one hour to solidify. The antibiotic paper disc containing Ceftriaxone (30 µg), Ceftazidime (30 µg), Amikacin (5 µg), Ciprofloxacin (5 µg), Gentamicin (10µg), Chloramphenical (30 µg), Amoxylin (10 µg), and Tetracycyline (30 µg) (Oxoid UK) were aseptically placed on the surface of the molten Mueller Hinton agar and allowed for 30 minutes to pre-diffuse. The set up was carried out in triplicate for each isolate, with a control plate containing no antibiotic disc. These were incubated for 18-24 h at 37 °C, after which the diameter of zone of inhibition was taken and the results were interpreted using standard interpretative charts as recommended by the Clinical Laboratory Science Institute (CLSI, 2017). Multiple antibiotic resistance was indicated by resistance to a minimum of three different antibiotics

Statistical Analysis

One way analysis of variance was used to analyze data. Significant difference between means (P≤0.05) was considered with Duncan's New Multiple Range Test using SPSS version 2017.

RESULT

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Toxicity Effect of *Tetracarpidium conophorum* Seed Extract

The result of acute toxicity is shown in Table 1. There were no signs of toxicity such as paw licking, sleeping, reduced activity, respiratory distress observed in mice and there were no mortality.

Effect of *Tetracarpidium Conophorum* Seed Extract in Mice Body Weight

The result of the effect of *Tetracarpidium conophorum* seed extract on body weight of mice is shown in Table 2. The body weight of mice in group 5 (infected and not treated) was reduced from 19.71g on day 0 to 18.17g on day 5 and there was insignificant increment from day 2 to day 3 (18.57 to 19.57). Changes occurs in the weight of mice in group 1 (mice treated with 200mg/kg) from 19.71g to 14.50g wherein significant increment was observed only from day 3 to 4 (18.67g to 16.00g), group 2 (i.e mice treated with 400mg/kg) 19.71g to 16.00g) wherein insignificant increment was perceived only from day 3 to 4 (21.319 to 21.00g). However, group 3 had significantly increase in weight on the first day of treatment (18. 41g to 20.42g) then the increment and decrement from a day to the next day tends to be insignificant until from day 4 to day 5 when a significant decrement in weight was observed (20.42g to 17.80g). It should also be noted that the reduction in weight between days 0, 1 and 2 were also significant with that of day 5, hence we could conclude a reduction in weight in group 3 also. It is only in group 4 that we observed a significant increase in weight of mice because the reduction in weight observed between day 2 (20.71g) and day 3 (20.14g) and between day 4 (20.43g) and day 5 (18.71g) were not significant.

Anti-typhoidal Effect of Ethanol Extract of *Tetracarpidium Conophorum* Seed on Typed and clinical isolates of *Salmonella ~~T~~typhi*

The ethanol extract of *Tetracapidium conophorum* were all resistant to typed isolates of *Salmonella typhimurium* and clinical isolates of *Salmonella typhimurium* at 6.01±0.10 mm which was the zone diameter of the boreholes on the prepared agar as shown in Table 3.

Antibiotic Sensitivity Pattern of Typed and Clinical *Salmonella ~~T~~typhi*

Ciprofloxacin (5 µg) had the highest zone of inhibition at 32.50±2.50 mm against typed *Salmonella typhimurium* while tetracycline (30 µg) had the least of 15.50±0.50 mm against the same bacteria. Ciprofloxacin (5 µg) also had the highest of 32.50±2.50 mm against clinical *Salmonella typhimurium* while ceftazidime (30 µg) had the least of 6.010±1.00 mm against the same bacterial organism as shown in Table 4.

Table 1: Acute toxicity of *Tetracarpidium conophorum* ethanol seed extract

Groups	Dosage (mg/kg)	Mortality	Mortality (%)	Signs of Toxicity
1	800	0/4	0	Nil
2	400	0/4	0	Nil
3	200	0/4	0	Nil
4	100	0/4	0	Nil

Table 2: Profile weight of experimental mice

	Weight of Mice (g)				
Days	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Before Treatment	19.71 ±0.74	19.71±0.92	18.14±0.59^{de}	18.00±0.44^{bcd}	19.71±0.60
1	17.42±1.49^{a}	21.00±1.69^{a}	20.42±0.75^{ad}	20.14±1.26	19.00±0.49
2	17.17±1.60	21.17±2.02^{b}	20.29±0.87^{b}	20.71±0.75^{ab}	18.57±1.29
3	18.67±0.67^{a}	21.33±2.06^{c}	19.86±0.91	20.14±0.55^{c}	19.57±0.95
4	16.00±0.00	21.00±2.19	20.42±0.43^{ce}	20.43±0.43^{d}	19.17±1.01
5	14.50±0.50	16.00±1.87^{abc}	17.80±0.37^{abc}	18.71±0.57^{a}	18.17±0.91

Data are presented as Mean ± S.E (n<=7). Values with the same superscript letter(s) along the same column are significantly different (P≤0.05).

Legend: Group A = 200mg/kg, Group B =400mg/kg, Group C = 800mg/kg, Group D = infected and treated with chloroquine, Group E = not infected

Table 3: Inhibition Zone Diameters (mm) of the Ethanol Extract of *Tetracapidium Conophorum* on Clinical and Typed *Salmonella Typhi*

mg/ml	Zone of inhibition (mm)	
	1	**2**
1000	6.01±1.12[a]	6.01±1.02[a]
500	6.11±1.02a	6.01±1.02[a]
250	6.01±1.22[a]	6.01±1.02[a]
125	6.20±1.02[a]	6.01±1.02[a]
62.5	6.21±1.22[a]	6.01±1.02[a]
31.3	6.22±1.12[a]	6.01±1.02[a]
15.6	6.01±1.02[a]	6.01±1.02[a]

Superscripts carrying the same alphabet are not significantly different. Mean +/- standard error

1= *S. typhi* Clinical
2= *S. typhi* typed

Table 4: Antibiotic sensitivity pattern of clinical and typed *Salmonella typhi* isolates

Antibiotics	Typed *S. typhi*	Clinical *S. typhi*
	Zone of inhibition (mm)	Zone of inhibition (mm)
Ceftriaxone (CRS)	27.00[a]±1.00	26.50[a]±1.00
Gentamiycin (CN)	26.00[a]±1.00	26.00[a]±1.00
Amikacin (AK)	25.00[a]±5.00	20.50[a]±1.00
Ciprofloxacin (CIP)	32.50[a]±2.50	32.50[a]±2.50
Chloramphenicol (CLR)	27.50[a]±2.50	27.50[a]±2.50
Amoxylin (AML)	20.50[a]±4.50	23.50[a]±4.50
Ceftazidime (CAZ)	15.50[a]±9.50	6.010[a]±1.00
Tetracycline (TE)	15.50[a]±0.50	15.50[a]±1.50

Superscripts carrying the same alphabet are not significantly different at $p \leq 0.05$. Mean +/- standard error

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DISCUSSION

~~The essence of t~~This study ~~is to ascertain~~shows the *in-vivo* anti-plasmodial activity of ethanol *Tetracarpidium conophorum* seed extract and its anti-typhoidal activity *in-vitro.* The result of the acute toxicity of *Tetracarpidium conophorum* ethanol extract on mice observed in this study is parallel the findings of Hodge and Sterner (2005) which deduced that the acute toxicity test of the seed extract at different concentrations which revealed no mortality rate and behavioural signs of toxicity on all the experimental mice is expected. This indicates that the extract is nontoxic. This is in agreement with Hodge and Sterner Toxicity Scale which reported that any chemical exhibiting LD50 above 1000 mg/kg is practically non-toxic.

Decrease in mice weight observed on the second day of infection with the *Plasmodium* parasite, could presumably be due to the decrease in food intake and disturbed metabolic functions associated with malaria infection. This corroborates the findings of Basir *et al.*, (2012). The decrease in weight that was observed in mice treated with different concentration of the seed extract could be due to the phytochemical repertoire which is akin to the anti-nutritional factors in *T. conophorum* (Edem *et al* 2017). On the other hand, this decrease in mice weight could have been probably due the reason advanced that tannins reduce the bioavailability of proteins and protein value of foods (Ford and Hewith, 2010). Malaria, according to Basir *et al.* (2012) was reported to have induced low temperature in mice. This reason could have probably been responsible for the observed decrease in the temperature of the malaria infected experimental mice in this study. In addition, the decrease in temperature observed in the untreated *Plasmodium berghei* infected mice could be attributed to the debilitating effects of malaria on the host (mice) which could have brought loss of body heat due to the large surface area to body mass ratio of small animal like mice. This is in agreement with World Health Organisation (2011). Increase in temperature observed in mice administered with different concentrations (200 mg/kg, 400 mg/kg and 600 mg/kg) of *T. conophorum* seed extract agrees with findings of Ford and Hewith (2010) who reported an increase in temperature of malaria infected mice treated with the extract of *Russelia equisetiformis*is and attributed the reason to be due to the bioactive component of the seed extract.

The high susceptibility of chemotherapeutic agents in this study is due to the emerging changing status of *Salmonella typhi* sensitivity to antibiotics. This is in contrast to the resistance to multiple antimicrobial agents in *Salmonella typhi* which has been a major problem, especially in Asia (Pary *et al.,* 2002). The emergence of antibiotic resistant strains of the bacteria is closely related to the irrational use of antibiotics in treating human salmonella infection. However, due to development of resistant to Chloramphenicol use has declined significantly, particularly in developed countries where third generation Cephalosporins or Ciprofloxacin are used preferably. Therefore the sensitivity pattern of *S.typhi* is

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changing and there is re-emergence of sensitivity to Chloramphenicol but rising resistance to Ciprofloxacin (Gautam *et al*., 2015).

CONCLUSION

The ever increasing global spread of drug resistance to the available anti-malarial drugs is a major concern and requires innovative strategies to control the disease. This study has revealed the anti-plasmodial efficacy of *Tetracarpidium conophorum* seed. Findings have shown that the ethanol seed extract of *T. conophorum* relatively possess anti-plasmodial and anti-typhodial activities compared to the positive antibiotic susceptibility of *Salmonella typhi*. Auxiliary evaluation must be conducted to establish the anti-typhoidal activity of ethanol *T. conophorum* extract *in-vivo*

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DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

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Comment [PA13]: Please double check the journal reference style.

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