International Journal of Scientific Research in Biological Sciences
Vol.10, Issue.3, pp.53-57, June 2023
E-ISSN: 2347-7520
Available online at: www.isroset.org

Research Paper

Antitrypanosomal Potential of Wonderful Kola Methanolic Extract Against *Trypanosoma brucei brucei*

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Received: 22/Mar/2023; **Accepted**: 29/Apr/2023; **Published**: 30/Jun/2023

Abstract— Several pharmacognostic properties of wonderful kola plant has necessitated the need for a high-throughput identification of bioactive compounds present in this plant which could serve as lead compounds in developing potent and novel trypanocidal agents. Trypanosomiasis is a protozoal disease known as sleeping sickness which is ranked among top priority tropical infections. The aim of the current study was to identify the bioactive compounds present in methanolic extract of wonderful kola and determine its antitrypaosomal activity. Photochemical screenings and Gas Chromatography Mass Spectrometric Analysis of the plan extracts was carried out to determine the bioactive compounds. A total of 12 adult rats, 7 weeks old of both sexes were randomly divided into six groups (1, 2,3,4,5 and 6) of 2 animals each. All the Groups were intraperitoneally infected with 0.1 mL of blood containing 10^6 Trypanosomes/mL. Groups 1- 4 received methanolic extract of wonderful kola intraperitoneally at daily doses of 0.01, 0.1, 1.0 and 10 mg/mL/kg body weight, while Group 5 received standard trypanosomal drug of 7.86 mg/kg (suramin dose) which served as the positive control and Group 6 was infected without any treatment (negative control). Different pathological parameters in terms of parasitemia, temperature, weight and percentage of survival were all evaluated. Some of the compounds identified include: saponins, tanins, glycosides, terpenoids, steroids, alkaloids, flavonoids, benzendodecanoic acid, methyl tetradecanoate, hexadecanoic acid, diisooctyl adipate, cycloheptatien. Among the animals treated with methanolic extract of wonderful kola, there was decrease in the level of parasitaemia, decrease from higher to normal body temperature, increases in body weight and survival rate. In conclusion, Wonder Kola contains important bioactive compounds which are relatively safe and could be regarded as potential Trypanocidal agents in the treatment of trypanosomiasis.

Keywords— Wonderful kola Extract, *A*ntitrypanosomal activity, *Trypanosoma brucei brucei*, Parasitaemia, Suramin

1. Introduction

Trypanosomiasis is a protozoal disease known as sleeping sickness which is ranked among top priority tropical infections [1]. It is chronic upon both humans and susceptible mammals leading to fatalities and high economic loses [2]. This haematozoan disease is associated with anemia, loss of body condition, pyrexia, lacrimation, pallor of the mucus membrane, weight loss, infertility, abortion and sometimes death [3].The clinical manifestation of this zoomastigophoran disease is influenced by the host immune status, trypanosome species and strain which is characterized by the intermittent fever [1].

B*uchholzia coriacea* seed commonly called wonderful kola belongs to the plant family *Capparaceae* [4].The use of wonderful kola in traditional medicine practices have been credited to some bioactive compounds present in the seed, which were reported to have diverse pharmacognostic properties [5], as such wonderful kola has enjoyed popularity in recent times due to the high cost of some available conventional drugs which may not be available and affordable by most people in rural communities as well as the development of resistance to drugs. This study is aimed at identifying the bioactive compounds in wonderful kola and further evaluates its antitrypanosomal activity.

2. Related Work

Several pharmacognostic properties of wonderful kola plant has necessitated the need for a high-throughput identification of bioactive compounds present in this plant which could serve as lead compounds in developing potent and novel trypanocidal agents [5].Wonderful kola has been considered

as a brain food which promotes memory with multiple medicinal values, used in the treatment of many diseases such as; diarrhoea, malaria, rheumatism, ulcers, worm infection, asthma, cough, diabetes, hypertension, psychiatric disorders, and many others but no or very little information was recorded from the existing literature about its antitrypanosomal potential [1]. Trypanosomiasis been among the top priority neglected tropical disease with only few therapeutic options, causes immune suppression in the host, which result to a serious illness in human and direct losses in meat production and milk yield in animals [3]. It is estimated that about 60 million people are at risk of the disease, of which only 3.5 million are under surveillance in endemic countries [2]. The management of the disease is principally based on vector control, vaccination and use of trypanocidal drugs [1]. Consequently, production of vaccine is hindered by antigenic variation exhibited by the parasite and vector control strategy is associated with many challenges, while use of drug control is always resulted to some significant side effects [1,3]. Therefore, vigorous efforts are recommended towards a continuous search for safer, potent and affordable drugs of plants origin to actualize effective control and eradication of the menace. It is therefore against this bedrock, that this research was conducted in order to evaluate the in vivo antitrypanosomal potential of wonderful kola methanolic extract against *Trypanosoma brucei bruei.*

3. Methodology

Ethical Approval
Ethical approval was obtained from Bayero University Kano ethical committee for the care and use of animals. All experimental protocols were conducted with strict adherence to guidelines established by the committee for the care and use of animals.

Plant Material
Fresh seeds of wonderful kola were purchased from Ilorin state, Nigeria and were authenticated at the herbarium, Department of Plant Science, Bayero University, Kano-Nigeria. Voucher specimen number 2471 was deposited at the herbarium. The plant was washed and grinded using hand grander, and then shed dried at room temperature for 7 days. The dried wonderful kola were pounded into fine powder using mortar and pestle, and then stored in a dry and sterile container [6].

Extraction and Phytochemical Screenings
The wonderful kola seed powder was extracted by cold maceration method using methanol according to the methods of [7]. Exactly 300 g of the plant powder was weighed and dissolved in 900 mL of methanol at the ratio of 1:3 powdered seed to methanol. The mixture of powdered seed and methanol were allowed to stand for 2 days with regular shaking, it was then filtered using muslin cloth and left to evaporate in a water bath at 40^{o}C. The phytochemical constituents (saponins, tannins, glycosides, terpenoids, steroids, alkaloids and flavonoids) of the plant were determined by the standard screening method of [8].

Gas Chromatography Mass Spectrometric (GC-MS) Analysis
The powdered form of the wonderful kola extract was re-constituted in a methanol and loaded in to the GC-MS machine for the determination of the chemical constituents [9].

Experimental Animals
A total of 12 adult rats of approximately 7 weeks old weighing between 140-200g each, were used in this experiment. They were obtained from the Animal House, Department of Human Anatomy, Bayero University Kano. They were allowed to acclimatize for 14 days in research laboratory, where the experiment was conducted. The rats were housed under standard hygienic conditions in plastic cages, fed with commercial feed (Vital feeds LTD, Kano, Nigeria) and given access to clean water ad libitum which was maintained accordingly by the animal curator.

Parasites
Trypanosoma brucei brucei (Federi strain) was used for this research, it was obtained from Nigerian Institute for Trypanosomiasis and Onchocerciasis Research, Vom, Plateau State, Nigeria. The parasite was isolated from cattle in 2018, identified as *T. b. brucei* and stabilized by four passages in rats before storage in liquid nitrogen. It was confirmed and characterized by the standard trypanosome detection methods of [11] as described by Nakayima [12]. The parasite was maintained by serial passages in donor rats and infected blood from the donor rat was collected at peak parasitemia by tail bleeding. The infected blood was diluted in physiological saline and then inoculated into the peritoneal cavity of the experimental rats.

Experimental Design and Inoculation of Experimental Animals with Trypanosomes
The 12 experimental rats were randomly grouped into six groups (1 – 6) of 2 animals each. All the Groups (1-6) were intraperitoneally infected with 0.1 ml of blood containing approximately 10^{6} Trypanosomes/mL. Treatment began the day parasites were first detected by microscopy in the blood stream (day 4) and continued up to day 14 when the entire negative control died. Groups 1- 4 received methanolic extract of wonderful kola intraperitoneally at daily doses of 0.01, 0.1, 1.0 and 10 mg/ml/kg body weight respectively, while Group 5 received standard Trypanosomal drug of 7.86mg/kg (suramin dose) which served as the positive control and Group 6 was infected without any treatment (negative control).

Monitoring Different Pathological Parameters
Parasitaemia was monitored daily using the rapid matching method of [13]. By preparing a wet mount from the peripheral blood by means of tail-bleeding, in which a drop of blood was placed on a clean glass slide and covered with cover slip. Each slide was prepared separately and observed. The number of parasites was determined microscopically by counting the parasite in each field and matching with standard chart. Body temperature of all the experimental animals was measured daily, each rat was gently caught and a digital

thermometer was inserted 3 cm into the anus of each rat and at the sound of a beep, the thermometer was immediately withdrawn and values obtained were recorded as described by [14], while daily body weight of the experimental rats was determined using a digital weighing balance and percentage of survival was monitored daily and expressed as number of survivor divided by total initial number in the group multiplied by 100% [9].

4. Results and Discussion

Photochemistry

The result of the study revealed the presence of saponins,tannins, glycosides, terpenoids, steroids, alkaloids and flavonoids in the methanolic extract of wonderful kola (Table 1).

The Gas Chromatography Mass Spectrophotometry analysis revealed that Benzene was the first to elute with retention time of 8.44 and area of 0.83 followed by Dodecanoic acid (9.87, 1.23%); Methyl tetradecanoate (10.49, 1.73%); Hexadecanoic acid (13.72 , 3.37%); Diisooctyl adipate (22.25, 79.67%); Cycloheptatrien (29.44, 12.54%) and Isopropanol (29.96, 0.64%) (Table 2).

Antitrypanosomal activity of the plant extract

The mean parasitaemia of the experimental animals infected with *Trypanosoma brucie brucie* is presented in Figure 1. For all the six groups, the parasitaemia became patent by 4 days post infection. There was an increased in parasitaemia level for group 6 (negative control) throughout the experiment. Also a little increase in parasitaemia was observed in group 1 (rats treated with 0.01 mg/kg methanolic extract of wonderful kola [WE]) while little or no increase in parasitaemia was observed in group 2 (rats treated with 0.1 mg/kg WE) and group 3 (rats treated with 1 mg/kg WE). More so, complete elimination of parasitaemia was observed in group 4 (rats treated with 10 mg/kg WE) and group 5 (positive control [rats treated with Suramin]).

The Mean temperature of the experimental animals infected with *Trypanosoma brucie brucie* is presented in Figure 2. There is stationary increases in the mean body temperature in group 6 (negative control) up to (40˚C) at Day 14 post infection. Group 1 (rats treated with 0.01 mg/kg WE), group 2 (rats treated with 0.1 mg/kg WE), group 3 (rats treated with 1 mg/kg WE), group 4 (rats treated with 10 mg/kg WE) and group 5 (positive control [rats treated with Suramin]) maintained a standard body temperature of (36- 37˚C) from Day 1-Day 3, after which the temperature reduced to (34˚C - 36˚C) at Day 14 post infection respectively.

Figure 3 shows the mean body weight of the experimental animals. There was a slight increase in mean body weight in Group 1 (rats treated with 0.01 mg/kg WE), group 2 (rats treated with 0.1 mg/kg WE), group 3 (rats treated with 1 mg/kg WE), group 4 (rats treated with 10 mg/kg WE) and group 5 (positive control [rats treated with Suramin]), while a clear trend of decreasing in mean body weight was seen in Group 6 (negative control) respectively (Figure 3).

Percentage Survival of the experimental Animals

The Percentage Survival of the experimental Animals is shown in Figure 4, there was 100% Survival throughout the experiments in Group 1 (rats treated with 0.01 mg/kg WE), group 2 (rats treated with 0.1 mg/kg WE), group 3 (rats treated with 1 mg/kg WE), group 4 (rats treated with 10 mg/kg WE) and group 5 (positive control [rats treated with Suramin]), while 0 % survival was observed and recorded in Group 6 (negative control) respectively.

Table 1. Phytochemical Constituents of Methanolic extract of wonderful kola

Phytochemical Constituents	Indications
Saponins	+
Tanins	+
Glycosides	+
Terpenoids	+
Steroids	+
Alkaloids	+
Flavonoids	+

Key: + = present

Table2. Bioactive Compounds detected by Gas Chromatography Mass Spectrophotometry in methanolic extract of wonderful kola

Chemical Compounds	Retention Time (Minutes)	Area (Percentage)
Benzene	8.44	0.83
Dodecanoic acid	9.87	1.23
Methyl tetradecanoate	10.49	1.73
Hexadecanoic acid	13.72	3.37
Diisooctyl adipate	22.25	79.67
Cycloheptatrien	29.44	12.54
Isoproponol	29.96	0.64

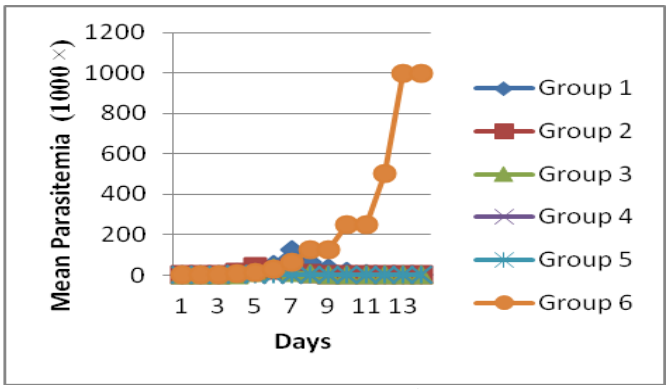

Figure1. Mean Trypanosome Parasitaemia of the experimental animals within 14 days period

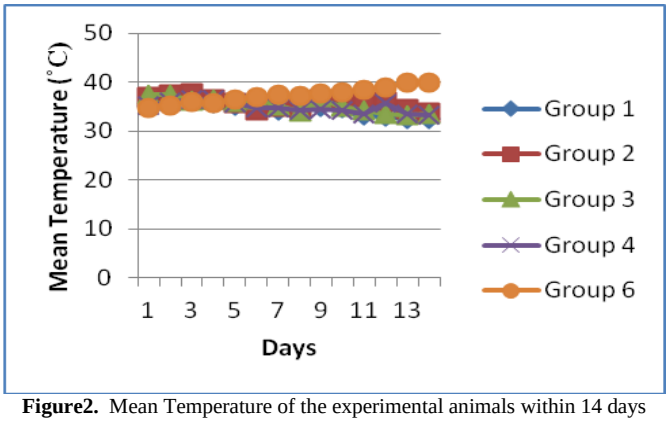

Figure2. Mean Temperature of the experimental animals within 14 days period

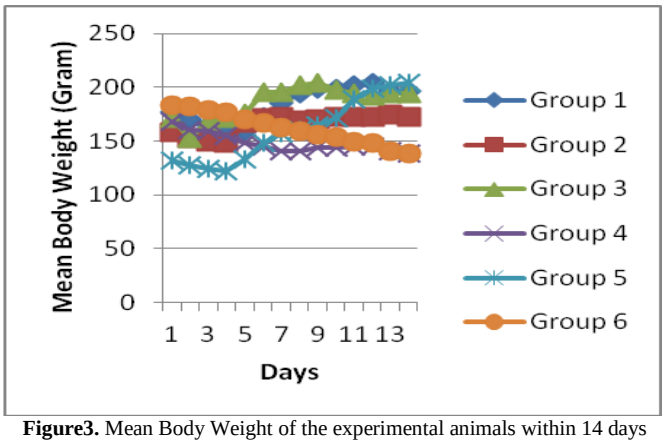

Figure3. Mean Body Weight of the experimental animals within 14 days period

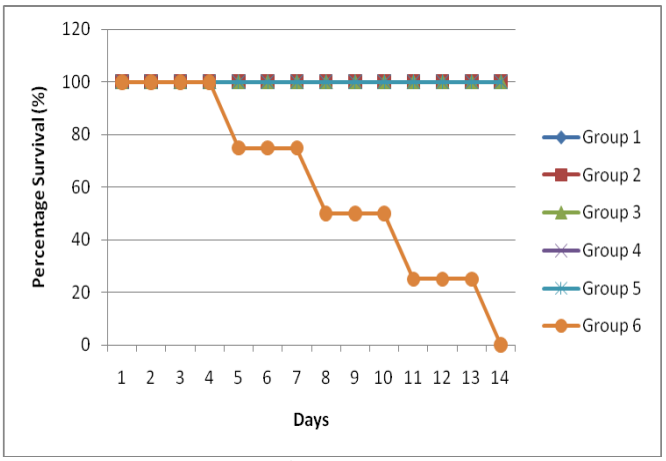

Figure4. Percentage Survival of the Experimental Animals within 14 days period

Discussion

The study findings revealed the presence of some important phytochemiacals in the methanol extract of wonderful kola that include saponins, tannins, glycosides, terpenoids, steroids, alkaloids and flavonoids. It has been reported by earlier studies that phytochemicals including tannins, saponins and alkaloids among others, are important components of natural products and they possess numerous bioactive activities which are well documented [15]. More so, these bioactive substances confer resistance to plants against wide range of parasites and other microorganisms [5,15]. The result of this study is in line with the finding of [4,5,15] who also identified tannins and steroids in wonderful kola extract.

In comparison with earlier reports, the present study also identified important compounds using the GC-MS that have many uses in medical field and possessed interesting biological activity against certain illness and pathogens. For instances, the reported antitrypanosomal, antioxidant and antimicrobial activity of Hexadecanoic acid may suggest the rationale for the traditional use of wonderful kola [16]. Benzoic acid compound was reported to have antimicrobial effect, while Hexadecanoic acid is known to possess variety of pharmaceutical value, such as anti-oxidants, pesticidal and nematocidal effects hence possible potent antitrypanosomal agents [17].

The pathogenesis and clinical manifestation of trypanosomiasis is always associated with; increase in parasitaemia, increases in body temperature, decrease in body weight and even death if there is no drug intervention [9]. Thus, any antitrypanosomal agent is expected to show the reverse pattern in any of the case [18]. In this study, little or no increase in parasitaemia, decrease from higher to normal body temperature and increase in body weight were all observed and recorded in Groups 1 – 5 that were administered the Wonder kola methanol extract in different concentrations, while the reverse pattern were observed and recorded in Group 6 (negative control). Therefore, these results indicated the trypanocidal potential of methanol extract of wonderful kola, and this report is in line with the work of [15]. The trypanocidal activity of methanol extract of wonderful kola observed in this study might be attributed to the action of the individual class compounds isolated in the extract, or to the synergistic effect that each class of compounds exerted to give the observed antitrypanosomal activity as explained by [19].For example, tannin coagulate the wall proteins of the trypanosomes, saponins facilitates the entry of toxic material or leakage of vital constituents from the cell, flavonoids inhibit the activity of enzymes by forming complexes with extracellular materials and soluble proteins, while alkaloids prevents nucleic acid synthesis and DNA interaction in combination with protein biosynthesis inhibition [17].

5. Conclusion and Future Scope

The study revealed that Methanolic extract of wonderful kola contains important phytochemical components and other bioactive compounds as potent trypanocidal agents. Therefore, these results indicated the *in vivo* antitrypanosomal potential of methanolic extract of wonderful kola aginst T*rypanosma brucie brucie*. Meanwhile further research is needed to elucidate the mechanisms by which genes mediate resistance to Trypanosomal drugs, so as to overcome the spread of drug resistance. Future research should also be carried out using different species and sub-species of trypanosome and other Higher animals that are natural host of Trypanosome (such as Goats, Pigs, Horses and Cattle) before adopting the result of this finding for laboratory use.

Conflict of Interest

The authors declared no conflict of interest.

Funding Source

This research received no external funding.

Authors' Contributions

F. A. Rufa'i researched literature and conceived the study. A. B. Yerima involved in protocol development, gaining ethical approval, and data analysis. H. H. Sani and F. A. Rufa'i wrote the first draft of the manuscript. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Acknowledgements

We gratefully acknowledge the efforts of all the contributors involved in this research. We personally appreciate the

expertise of Prof. M D Mukhtar of Microbiology Department, Bayero University Kano for his contribution towards the success of this publication.

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