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PHARMACOLOGICAL EVALUATION OF *GARCINIA KOLA* NUTS FOR ANTI-TRICHOMONAL ACTIVITY

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ABSTRACT

Treatment options for trichomoniasis are extremely limited. Newer drugs are therefore needed. Antitrichomonal effects of *G. kola* nuts were evaluated so that extracts with significant antitrichomonal activities can be standardized for use in phytotherapy of trichomoniasis. Powdered nuts were extracted with 100% methanol (**A**) and 50% methanol (**B**). The marc from **A** was further extracted in water to obtain (**C**). **A, B, C,** and fractions of **A** were screened against *Trichomonas gallinarum in vitro*. Antitrichomonal activities were in the order **A**> **B** > **C** at 24 h with LC_{50} and LC_{90} of 36.14., 50.12, 212.9 and 293.77, 535.29, 5355.4 (µg/ml) respectively. At 48 h, the order was **B**> **A** > **C** with LC_{50} and LC_{90} of 58.51, 139.55, 195.62 and 195.62, 1434.09 and 2887.29 (µg/ml) respectively. It is concluded that these extracts are sufficiently trichomonacidal and therefore potentially useful as therapeutic agents in the control of trichomoniasis.

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KEY WORDS

Trichomoniasis, antitrichomonal, tricomonocidal, *Garcinia kola*, *Trichomonas gallinarum*

INTRODUCTION

Trichomoniasis (also known as trichomonas vaginitis, trichomonas vaginalis, or trich) is an infection of the genital and urinary tracts, caused by a single-celled protozoan, trichomonas. It is the most common sexually transmitted disease, affecting about 170 million people worldwide each year. *Trichomonas* are found in the mouth, where they may contribute to gingivitis; in the intestine where they may be associated with diarrheal conditions and in the urethra and vagina, where they cause an inflammation and purulent discharge[1,2,3].

Current chemotherapy of trichonomiasis is based mainly on the nitroimidazole class of drugs, the most commonly used being metronidazole (Flagyl) and tinidazole (Tindamax). Although these drugs are reasonably efficacious, the chemotherapeutic armoury for trichomoniasis remains grossly inadequate. There is therefore the need for safe and efficacious drugs for the effective treatment of trichomoniasis.

The search for antimicrobial agents of natural origin has become very appealing with the increasing problem of drug resistance, more so when there is a global problem of emerging and re-emerging infectious diseases. Dichloromethane extract of the bark of *Cussonia holstii* Harm ex. Engl. (Araliaceae), a traditional medicinal plant used in Kenya has been reported to have a high anti-trichomonal activity. Using centrifugal partition chromatography, a pentacyclic triterpenoid, hederagenin, with IC_{50} of 2.8 µg/ml was isolated from this extract as the anti-trichomonal constituent of the plant[4].

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Garcinia kola nut, generally known as *Bitter kola* in Nigeria belongs to a family of tropical plants known as Guttifera[5]. *G. kola* is found in moist forest and grows as a medium size tree, up to 12 m high. It is cultivated and distributed throughout west and central Africa. It is commonly called "*Namijin goro*" in *Hausa, Orogbo* in *Yoruba* and "*Agbilu*" in *Igbo*. *G. kola* nuts are used in traditional medicine for various ailments such as sore throat and arrow "poisoning". Other medicinal uses include its use as purgative, antiparasitic, antimicrobial, antiviral, anti-inflamatory, antidote to the effects of *Strophantus gratus*, remedy for guinea-worm infection and for the treatment of gastroenteritis, rheumatism, asthma, menstrual cramps, bronchitis, throat infections, cure headache, relieve colic, chest colds, cough, and liver disorders[6,7,8,9]. The plant is also used as antidiabetic, antioxidant, and for the chemoprevention of aflatoxin B1 and antihepatotoxic activities[10,11,12,13,14].

The phytochemicals obtained from *G. kola* as documented in literature includes biflavonoids, xanthones, kolanone, ameakoflavone, 24-methylenecyclartenol, coumarine and prenylatebenzophenones[15]. Others include oleoresin[16], the chromanols, garcioic and garcinal[17].

This study was planned with the main objective of evaluating the bioactive potentials of extracts of *G. kola* nuts as antitrichomonal agents. We report here that aqueous fractions of 100% methanol extract and 50% methanol extract of *G. kola* seed are remarkably trichomonacidal with immense potential to add to the broad spectrum pharmacological applications of *G. kola*.

MATERIALS AND METHODS

Local Pigeon *Columba lavia* and the raw chicken eggs were purchased in the central market, Minna, Niger state, Nigeria. *T. gallinarum* were isolated from the throat of

local Pigeon, *C. lavia* using sterilized swab stick and saline. The *Trichomonas* in saline solution was multiplied by culturing in test tubes containing prepared egg slant and overlay (50 ml of ringer solution – NaCl, 6.5g; NaH_2PO_4, 0.01g; KCl, 0.14g; $NaHCO_3$, 0.2g; $CaCl_2$, 0.12g; glucose, 2.0g dissolved and made up to 1 litre with distilled water - + 1 ml of cow blood serum + 1ml of 10% glucose) and then incubated at 37 °C[3]. *Garcinia kola* seeds were bought in January 2008 from the Minna central market, Niger state Nigeria, identified by Mr. Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria with herbarium specimen number 13184 and then air dried in the laboratory, specimen having been deposited at the herbarium. The nuts were then powdered and kept in big envelopes until required for use.

(i) ***Preparation of Extracts:*** About 181.00 g each of the powdered plant material were cold extracted five times for six days with 5 x 200 ml 100% methanol (**A**) and 50% methanol (**B**). The marc from **A** was further extracted using 4 x 250 ml of water to obtain (**C**). The collected extracts were bulked and concentrated *in-vacuo* using rotary evaporator. The three extracts (**A, B and C**) of *Garcinia kola* were screened against *T. gallinarum .in vitro*.

(ii) ***Partitioning of the Crude Methanol Extract A:*** The crude Methanol extract (A, 21 g) was suspended in 150 ml water, partitioned and concentrated *in-vacuo* to yield *n*-hexane (**A_1**, 2.03 g), chloroform (**A_2**, 4.15 g), ethyl acetate (**A_3**, 6.06 g) and aqueous fractions. The aqueous phase spontaneously segregated into two components. There was a component in the liquid phase (A_4, 7.05 g) and also a whitish precipitate (A_5, 0.51 g).

(iii) ***Anti-trichomonal assay:*** The assay was done by taking 5mg of the crude methanolic extracts (100%-A and 50%-B) and aqueous extract (C) of *Garcinia kola* and the partition fractions of A (**A_1 - $_5$**) and dissolving each in 1 ml of dimethylsulphoxide (DMSO) and diluting serially to give 1000, 500, 250, 125, 62.5, 31.25, 15.625, 7.812 and 3.906 µg/ml concentrations. The same serial dilutions were prepared for the positive control using metronidazole as the standard drug. The inoculum (150 µL) was added into each well of the 96-well flat-bottom microwell plates and 50 µL of the test substance or metronidazole in its appropriate concentration was then added. The plates were thereafter incubated at 37 ºC and cell growth monitored at 24 and 48 h by counting their numbers under the microscope. For the negative control, 50 µL of overlay with DMSO was added to the 150 µL inoculum instead of the test substance[18]. The tests were repeated six times (N = 6) for the test agents and the controls while the lethal doses (LD_{50} and LD_{90}) were determined at 24 and 48 h using the Finney Probit analysis and Minitab14 statistical software[18].

RESULTS

1. Percentage yield of extracts:
   The percentage yield of the crude extracts were 19.78%- **A**, 19.87% **-B** and 8.96%-**C** while that of the partition fractions were 9.67%- **A_1**, 19.76% - **A_2**, 28.86% - **A_3**, 33.57% - **A_4** and 2.43% -**A_5**.
2. Antitrichomonal activities: Antitrichomonal activities varied between 24h and 48 h. At 24 h the antitrichomonal activities were in the order **A**> **B** > **C** at 24 h with LC_{50} and LC_{90} of 36.14., 50.12, 212.9 and 293.77, 535.29, 5355.4 (µg/ml) respectively, while at 48 h, the order was **B**> **A** > **C** with LC_{50} and LC_{90} of 58.51, 139.55, 195.62 and 195.62, 1434.09 and 2887.29 (µg/ml) respectively. While fraction A_1 had some activity at 24 h, at 48 h, it showed no activity at all. The details are presented in Table 1.

Table 1
Antitrichomonal activity of extracts of G. kola nuts

	24 hours		48 hours	
Fractions	**LC_{50}(µg/ml)**	**LC_{90}(µg/ml)**	**LC_{50}(µg/ml)**	**LC_{90}(µg/ml)**
A	36.14	293.77	139.55	1434.09
C	212.9	5355.4	195.62	2887.29
B	50.12	535.29	58.51	304.71
A_1	1152.0	11384	- Ve	- Ve
A_2	55.38	254.98	103.35	1749.0
A_3	0.00	0.00	$9.7x10^{7}$	- Ve
A_4	603.35	4628	43.66	166.4
A_5	411.57	5884.4	33.66	164
MZ	14.04	61.8	14.04	61.37

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***A** = 100% methanol extract, **B** = 50% methanol extract, **C**, = aqueous extract of marc from **A**, **A_{1-5}** = partition fractions of **A** into hexane (A_1), chloroform (A_2), ethyl acetate (A_3), aqueous phase, suspension (A_4), precipitate (A_5), **MZ**: = Metronidazole, -Ve = no effect.*

DISCUSSION

The cidal effects of A and B at 24 h and 48 h (Table 1) show that A acts faster than B and therefore, potentially better than B in the treatment of acute trichomoniasis while B is potentially better than A in the treatment of chronic trichomoniasis. Combining B and A_5 may be very effective in combination therapy. The very good activity of fraction A_5 at 48 h but very poor activity at 24 h suggests its potential for use in both prophylaxis and therapeutic controls. It therefore means that A_5 both singly or in combination with B can be standardized and packaged into phytomedicine for effective treatment of trichomoniasis. This is more so as toxicity may not be a serious problem since *G. kola* snuts are widely consumed at social gatherings and in places of work and homes in Nigeria. *G. kola* has been taken for ages and found to be non toxic and found to have a lot of pharmacological properties[8,12,14,18].

It is clear that neither A_5 nor B is as good as the standard drug, metronidazole but these extracts have the advantage of being natural and therefore will be more readily acceptable which is important for patients' compliance, and there is less likelihood of resistance problem coming up early. Moreover with *Garcinia kola* extract, the risk of cellular damage, including DNA breakage and subsequent cell death and resistivity[12] may not exist.

The lack of activities in hexane and ethyl acetate fractions is an indication that the bioactive constituent(s) is/are polar, more so as the aqueous fraction, A_5 showed the best activity. This is reinforced by the relatively good activity of the chloroform fraction. The anti-trichomonal activity reported here has added to the broad spectrum pharmacological applications of the plant.

CONCLUSION

Garcinia kola methanol extracts (100%-**A** and 50%-**B**) have pharmacological activities against *T. gallinarum in vitro,* the aqueous fraction of **A** (**A_5**) having the highest antitrichomonal activity. Considering the

medicinal importance of this plant and resource within the reach of Nigerian people, **B** and **A_5** are sufficiently trichomonocidal to allow for their development into drugs for the effective treatment of trichomoniasis. .

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