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In vitro anti-infective and antioxidant activities of *Garcinia cola* Heckel and *Morinda lucida* Benth

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***Garcinia cola* also known as "bitter cola" (Guttiferae) is a plant with a wide usage of its parts for various medicinal purposes. The seeds are chewed as aphrodisiac and for the treatment of coughs, dysentery and liver inflammation. *Morinda lucida* (Rubiaceae) commonly called "great morinda" has been shown to have antimalarial and anti-pyretic activities. This study aimed at evaluating the anti-infective and antioxidant properties of *G. cola* and *M. lucida* and to justify their folkloric uses. Ethanol extracts of the stem barks of *G. cola* (GCB) and *M. lucida* (MLB) were evaluated for their antimicrobial, anthelmintic and antioxidant activities. Antimicrobial activity was evaluated by determining the minimum inhibitory concentrations (MIC) using the micro broth dilution method against strains of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Candida albicans*. Anthelmintic activity was evaluated by determining the effects of the extracts on the paralytic and death times of *Pheretima posthuma* at concentrations of 50, 20 and 10 mg/mL using piperazine citrate (PZN) (15 mg/mL) and albendazole (ABZ) (20 mg/mL) as references. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity using ascorbic acid (ASA) as reference standard. The results reveal that the extracts from both plants demonstrated antimicrobial activity with MIC values ranging from 50 to 80 mg/mL and 10 to 30 mg/mL for GCB and MLB, respectively. Both extracts also demonstrated a concentration dependent anthelmintic activity with decrease in paralytic and death times upon an increase in extract concentrations. GCB and MLB extract showed antioxidant activities with IC_{50} values, 6.830 and 342.1 µg/mL, respectively. Phytochemical screening of both extracts revealed the presence of tannins, glycosides, alkaloids and flavonoids. These findings may justify the folkloric uses of these plants.**

Key words: *Garcinia cola,* antioxidant, *Morinda lucida*, antimicrobial, anthelmintic.

INTRODUCTION

The use of medicinal plants to manage various ailments affecting humans have been in existence since ancient times. Studies have shown that in Africa, about 80% of the population rely on medicinal plants for the

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management of various ailments (Agyare et al., 2009). It is therefore important that such plants should be investigated to better understand their properties, safety and efficacy.

Morinda is a genus of flowering plants of the family, Rubiaceae (Umberto, 2000). In Ghana, the plant is used in managing ailments such as diabetes, hypertension, cerebral congestion, dysentery, stomach-ache, leprosy and gonorrhoea. Traditionally, the stems are used to treat piles while the leaves are used to treat fever (CSIR-FORIG, Ghana, 2017). Extracts of the plant have shown anti-inflammatory, febrifuge and pain reducing activity as well as antimalarial activity (Dalziel, 1973; Awe and Makinde, 1998). Methanol and ethanol extracts of the leaves of *Morinda lucida* have also been shown to possess antidiabetic activity (Bailey and Day, 1989). The leaves of the plant have also demonstrated trypanocidal activity (Asuzu and Chineme, 1990).

Garcinia cola (Guttiferae) is a multipurpose tree crop with increased value for the medicinal use of its parts. It is known as a wonder plant since every part of it has a medicinal importance. The seeds are chewed as an aphrodisiac or used to cure cough, dysentery, or chest colds in herbal medicine (Irvine, 1961). The latex or the gum is used internally against gonorrhoea and applied externally on fresh wounds (Iwu, 1989). The sap is used in curing parasitic diseases. The stem is used to produce bitter chewing sticks which is chewed chiefly as a masticatory to set an action of nervous alertness and has also been proven to exhibit pharmacological uses in treating coughs and throat infections (Farombi et al., 2005). *G. cola* serves as a source of raw material in the pharmaceutical industry; the raw stem bark can be used as a purgative, the powdered bark applied on malignant tumours (Iwu, 1989). *G. cola* exhibits purgative, antiparasitic, anti-inflammatory, antibacterial and antiviral properties (Ogunmoyole et al., 2012). Biflavonoid isolates, kolaviron, extracted from *G. cola* seeds were tested on streptozotocin (STZ)-diabetic rats. This study confirmed that cardiac, renal and hepatic function indices were significantly elevated during STZ-induced diabetes and that oral administration of kolaviron reduced the levels of some of the indices. Therefore, kolaviron may offer protection for tissues of animals during diabetes (Adaramoye, 2012). *G. cola* seeds have been reported to have an anti-inflammatory activity (Olaleye et al., 2000).

Although a lot of work has been done on the other parts of these plants, very little research has been conducted on the stem bark of these plants. This study therefore aims at evaluating the anti-infective and antioxidant properties of these plants and to justify or otherwise their folkloric uses.

METHODS

Plant collection

The stem bark of *G. cola* was obtained from the Forest Research Institute in Kumasi, Ghana while that of *M. lucida* was obtained from the physique garden of the Faculty of Pharmacy and Pharmaceutical Science, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana. Both samples were authenticated at the Department of Pharmacognosy, KNUST. The samples were sun dried for two weeks, cut into pieces and ground into powder using laboratory milling machine.

Extraction of plant material

A quantity of 200 g of the powder of each sample obtained was weighed and cold macerated using 1.0 L of 70 %v/v for 72 h. The bottles and their contents were placed on a Stuart mini orbital shaker and subjected to vigorous shaking hourly for 3 h. The supernatant solution obtained from each extract after 72 h was decanted into a clean beaker and filtered using a filter paper with the aid of a suction pump. The filtrates were then concentrated using a rotary evaporator (Buchi, Germany) at 40°C to obtain the crude extracts. The extracts obtained were then dried at 40°C in an oven until dry powdered extracts were obtained. The dried extracts were then kept in a desiccator until needed.

Phytochemical analysis

The presence of some secondary plant metabolites such as tannins, alkaloids, glycosides and flavonoids were tested using the dried crude extracts (Trease and Evans, 2002).

DPPH free radical scavenging activity

The free radical scavenging activity of the extracts were determined according to the method described by Agyare et al. (2015) using 1,1-diphenyl-2-picryl-hydrazyl (DPPH). MLB extract solutions of concentration 500, 1000, 1500 and 2500 µg/mL and GCB extract solutions of concentrations 1, 10, 30, 300 and 1000 µg/mL were prepared in test tubes with methanol. Solutions of the reference antioxidant (ascorbic acid) of concentrations 3.125, 6.25, 12.5, 25, 50 and 100 µg/mL were prepared in methanol. DPPH solution of concentration 0.002%w/v was also prepared in methanol in a dark room. Three millilitres of DPPH solution was added to 1.0 mL of each concentration of extract and reference antioxidant. The test tubes were then kept in the dark for 30 min after which the absorbance (A_1) of excess DPPH in both extracts and standard solutions were measured at 517 nm using a UV spectrophotometer (Jenway, USA). The absorbance (A_0) for a blank solution containing equal volumes of methanol and DPPH was also read and served as a control. The percentage of free radicals scavenged was calculated using the equation:

$$\% \text{ inhibition} = (A_o - A_I)/A_o \times 100$$

Inhibitory concentration (IC_{50}) was determined as the concentration of samples which scavenged 50% of free DPPH radicals.

Evaluation of antimicrobial activity

Test organisms

Clinical strains of *Staphylococcus aureus* and *Streptococcus pyogenes*, *Escherichia coli, Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans* were used for the studies. The organisms were obtained from the microbiology Department of Korle Bu Teaching Hospital, Accra, Ghana. The organisms were cultured in nutrient broth at 37°C for 24 h prior to the experiment.

Table 1. Antimicrobial activity of extracts.

Organisms	Minimum inhibitory concentration			
	Extracts (mg/mL)		Ciprofloxacin (µg/mL)	Ketoconazole (µg/mL)
	GCB	MLB		
Staphylococcus aureus	80	20	3.125	Na
Salmonella typhi	50	20	3.125	Na
Escherichia coli	50	10	3.125	Na
Pseudomonas aeruginosa	50	10	3.125	Na
Streptococcus pyogenes	50	10	3.125	Na
Candida albicans	70	10	Na	10

Na, No activity.

The turbidity of the actively growing broth cultures was adjusted with sterile distilled water to obtain a turbidity optically comparable to that of 0.5 McFarland Standard.

Micro-dilution assay

The minimum inhibitory concentration (MIC) was determined by the micro broth dilution method using 96 well microtitre plates (Eloff, 1998). A quantity of 50 µL of the double strength nutrient broth was used to fill each well. A volume of 5 µL of 24 h organism suspension was added as well as calculated volumes of the extracts, standard drugs (Ketoconazole and Ciprofloxacin) and sterile water to give a final well volume of 100 µL with varying extract and standard concentrations per well. The concentrations of extracts prepared ranged from 100 to 20 µg/mL. The microtitre plates were covered and incubated at 37°C for 24 h. A volume of 20 µL MTT (3-(4.5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) solution was added to the wells. The MIC was determined as the lowest concentration that inhibited the growth of the organisms which was indicated by the absence of purple coloration upon addition of the MTT solution.

Evaluation of anthelmintic activity

Collection of worms

Adult Indian earthworms were collected from the soil in a water logged area in Tema Community 10 and cabbage farms at Miotso near Central University, Ghana. The earthworms of length approximately 7 to 12 cm and width, 0.2 to 0.6 cm were used for the experiment due to their anatomical and physiological resemblance to human intestinal roundworm parasites and also because of easy availability; they are used extensively for the preliminary *in vitro* evaluation of anthelmintic compounds (Tiwari et al., 2011). The earthworms were washed with distilled water to rid them of debris.

Anthelmintic bio-assay

The worms were divided into eight groups each comprising of four earthworms. Ten millilitres of each extract solution of concentrations 10, 20 and 50 and mg/mL were prepared for both GBC and MLB using distilled water. Concentrations of 20 mg/mL albendazole and 15 mg/mL piperazine citrate were used as reference standards. All the samples and the standard drugs were freshly prepared before commencement of the experiments. The washed earthworms were placed in Petri dishes containing 10 mL of the respective formulations and concentrations. Observations were made for the time taken for paralysis and death of individual worms. Paralysis was noted when the worms ceased to move but were revived when shaken or placed in warm water at 50°C. Death was noted when the worms lost motility coupled with a fading away of their body colour. Normal saline was used as a negative control and the respective death and paralysis times were recorded (Bhawar et al., 2009).

Statistical analysis

All results and graphs were plotted and analysed using the Graph Pad Prism 5.0 for windows (Graph Pad software, San Diego, CA, USA).

RESULTS

Phytochemical screening

The phytochemical screening revealed the presence of glycosides, saponins, alkaloids, tannins and flavonoids in the extracts of MLB and GCB.

Antimicrobial activity

GCB and MLB extracts both demonstrated broad spectrum antibacterial and antifungal activity against the selected microorganisms. The antimicrobial activity was more profound in MLB as indicated in Table 1.

Antioxidant activity (free radical scavenging activity)

The antioxidant activity of GCB was highly profound as indicated in the IC_{50} value obtained (Table 2, Figure 1). MLB however showed poor antioxidant activity. The lower the IC_{50} value the more potent the antioxidant activity.

Anthelmintic activity

Both extracts demonstrated a concentration dependent anthelmintic activity as shown in Table 3.

Table 2. Inhibition concentration (IC_{50}) values of extracts and standard.

Sample	IC_{50} (µg/mL)
GCB	6.830
MLB	342.1
Ascorbic acid	2.929

Table 3. Anthelmintic activity of extracts.

Treatment	Concentration (mg/mL)	Groups	Time of paralysis (min) (mean±SEM)	Time of death (min) (mean±SEM)
0.9% Saline		1	Na	Na
ABZ	20	2	Na	1.06±0.14
PZN	15	3	2.10±0.01	Na
	50	4	18.17±0.03	24.34±0.21
MLB	20	5	58.41±0.24	85.42±0.01
	10	6	79.10±0.01	97.19±0.20
	50	7	39.29±0.12	54.29±0.01
GCB	20	8	41.18±0.05	75.15±0.18
	10	9	58.57±0.10	90.32±0.22

Na, No activity; ABZ, Albendazole; PZN, Piperazine citrate; MLB, *Morinda lucida* bark; GCB, *Garcinia cola* bark.

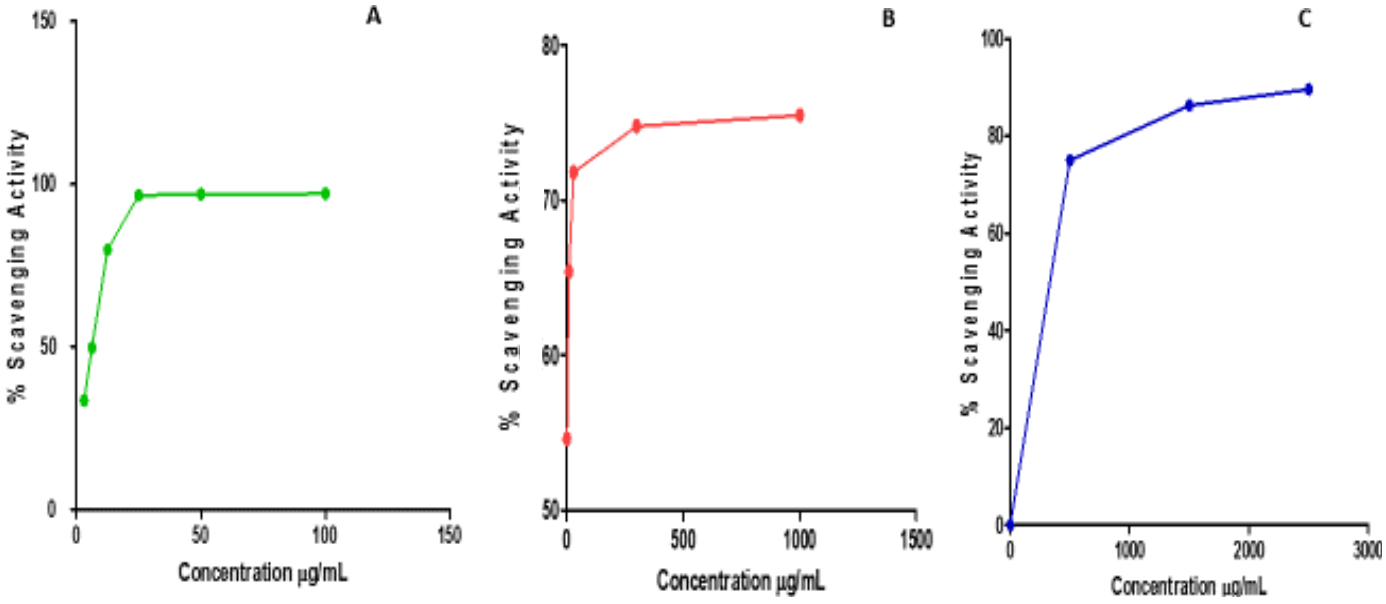

Figure 1. Free radical scavenging activity of extracts and standard. A, Ascorbic acid; B, GCB; C, MLB.

DISCUSSION

Phytochemical analysis of the extracts of both plants revealed the presence of tannins, saponin glycosides, anthraquinones, cardiac glycosides, alkaloids and flavonoids. Natural antioxidants are mainly obtained from plants rich in phenolic compounds such as flavonoids, phenolic acids and tocopherol (Ali et al., 2008). Phenolic compounds possess biological properties such as anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation,

anti-atherosclerosis, cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han et al., 2007). Flavonoids have also been found to have anti-microbial activity against a wide array of microorganisms (Pistelli and Giogi, 2012; Cushnie and Lamb, 2005).

The stem bark extract of *M. lucida* serves as a reservoir of bioactive phytochemical compounds. The results as shown previously confirm other studies conducted on this plant part (Fasola and Ogunyomi, 2005; Adomi and Umukoro, 2010). The *M. lucida* extract showed broad spectrum antibacterial activity as well as antifungal activity against selected microbes. The antimicrobial activity could be attributed to flavonoids and tannins present in the extracts (Gomes de Melo et al., 2010). The antioxidant activity exhibited could be attributed to the phenolic compounds in the plant (Kahkonen et al., 1999).

Various research works have been done on different parts of *G. cola* including the seeds, fruits, roots and leaves. However, very little research has been conducted on the stem bark of the plant. The stem bark extract demonstrated broad spectrum antibacterial activity as well as antifungal activity which could be attributed to the secondary metabolites as stated previously. Studies conducted by Ogunmoyole et al. (2012) on the seeds and fruit extract respectively have shown antioxidant activity. The stem bark extracts as used in this experiment also demonstrated antioxidant activity with IC_{50} value (6.830 µg/mL) almost comparable to that of ascorbic acid (2.929 µg/mL).

This gives an indication of the possible high level of phenolic compounds present in the stem bark hence the high antioxidant activity. *G. cola* bark extracts also demonstrated a concentration dependent anthelmintic activity with higher concentrations demonstrating better anthelmintic activity. Studies have largely attributed the anthelmintic activities of plants to the presence of tannins. Tannins are believed to interfere with the energy generation of the helminth parasite by uncoupling oxidative phosphorylation or by binding to free proteins in the gastrointestinal tract of the helminth. This eventually results in death of the parasite (Adu et al., 2015; Olusegun-Joseph et al., 2012).

Conclusion

Stem bark ethanol extracts of *M. lucida* and *G. cola* possess broad spectrum antibacterial and antifungal activity. Both extracts also possess antioxidant and concentration dependent anthelminthic activities. This could justify their use in folkloric medicine for the management of various ailments.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Adaramoye OA (2012). Antidiabetic effect of kolaviron, a biflavonoid complex isolated from Garcinia kola seeds, in Wistar rats. Afr. Health Sci. 4:498-506.

Adomi OP, Umukoro EG (2010). Antibacterial activity of aqueous and ethanol crude extracts of the rootbarks of *Alstonia boonei* and preliminary phytochemical test of *Morinda lucida*. J. Med. Plants Res. 4(8):644-648.

Agyare C, Asase A, Lechtenberg M, Niehues M, Deters A, Hensel A (2009). An ethnopharmacological survey and in vitro confirmation of ethnopharmacological use of medicinal plants used for wound healing in Bosomtwi-Atwima- Kwanwoma area, Ghana. J Ethnopharmacol 125:393-403.

Adu F, Apenteng JA, Akanwariwiak WG, Sam GH, Mintah DN, Bortsie EB (2015). Antioxidant and *in vitro* anthelmintic potentials of methanol extracts of barks and leaves of *Voacanga africana* and *Rauwolfia vomitoria*. Afr. J. Microbiol. Res. 9(35):1984-1988.

Agyare C, Baiden E, Apenteng JA, Boakye YD, Adu-Amoah L (2015). Anti-infective and Anti-inflammatory properties of *Portulaca oleracea* (L). *Donn.* J. Med. Plants Res. 2(1):1-6.

Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahuand A, Bora U (2008). Indian medicinal herbs as source of antioxidants. Food Res. Int. 41:1-15.

Awe SO, Makinde JM (1998). Evaluation of Plasmodium falciparum to *Morinda lucida* leaf extract sample using rabbit *in vitro* micro test techniques. Indian J. Pharmacol. 30(1):51-53.

Asuzu IU, Chineme CN (1990). Effects of *Morinda lucida* leaf extract on *Trypanosoma brucei brucei* infection in mice. J. Ethnopharmacol. 30(3):307-313.

Bailey CJ, Day C (1989). Traditional Plant Medicine as Treatment for Diabetes. Diabetes Care 12(8):553-564.

Bhawar GB, Bhalke RD, Lodha KR, Karmase BC, Londhe CD (2009). Phytochemical investigation and *in vitro* anthelmintic activity of *Bauhinia racemosa* (L) (Leguminosae). Pharmacology 1:300-303.

Council for Scientific and Industrial Research (CSIR) - Forestry Research Institute, Ghana (FORIG) (2017). Indigenous Knowledge on Forest Foods and Medicinal Plants in Ghana. Available at: http://csir-forig.org.gh/tikfom/

Cushnie TP, Lamb AJ (2005). Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents 26(5):343-356.

Dalziel JM (1973): The useful plants of West Africa. 1st ed. London: Crown Agents. pp. 403-404.

Gomes de Melo J, de Sousa Araújo TA, Thijan Nobre de Almeida e Castro V, Lyra de Vasconcelos Cabral D, do Desterro Rodrigues M, Carneiro do Nascimento S, Cavalcanti de Amorim EL, de Albuquerque UP (2010). Antiproliferative activity, antioxidant capacity and tannin content in plants of semi-arid north eastern Brazil. Molecules 15:8534-8542.

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64(8):711-713.

Farombi EO, Adepoju BF, Ola-Davies OE, Emerole GO (2005). Chemoprevention of aflatoxin B1-induced genotoxicity and hepatic oxidative damage in rats by kolaviron, a natural biflavonoid of Garcinia kola seeds. Euro. J. Cancer Prev. 14(3):207-214.

Fasola TR, Egunyomi A (2005). Nigerian Usage of Bark in Phytomedicine. Ethnomed. Bot. Res. Appl. 3:73-77.

Han X, Shen T, Lou H (2007). Dietary polyphenols and y their biological significance. Int. J. Mol. Sci. 8:950-88.

Irvine FR (1961). Woody plants of Ghana with special reference to their uses. Oxford University Press. pp. 693-695.
Iwu MM (1989). Food for medicine in dietary plants and masticatory as sources of biologically active substances, University of Ife press. pp. 303-310.
Kahkonen MP, Hopia AI, Vourela HJ, Rauha J, Pihlaja K, Kujala TH, Heinonen M (1999). Antioxidant activity plant extracts containing phenolic compounds. J. Agric. Food Chem. 47:3954-3952.
Ogunmoyole T, Olalekan OO, Fatai O, Makun JO, Kade IJ (2012). Antioxidant and phytochemical profile of aqueous and ethanolic extract of *Garcinia kola*. J. Pharmacogn. Phytother. 4(5):66-74.
Olaleye SB, Farombi EO, Adewoye EA, Owoyele BV, Onasanwo SA, Elegbe RA (2000). Analgesic and anti-inflammatory effects of Kolaviron (*A Garcinia* cola seed extract). Afr. J. Biomed. Res. 3:171-174.
Olusegun-Joseph TS, Ofodile LN, Oguntoku T (2013). *In-vitro* evaluation of anthelmintic activity of crude extracts of leaves of *Dalbergiella welwitschii*. Int. J. Pharm. Pharm Sci. 5(1):32-33.
Pistelli L, Giorgi I (2012). Antimicrobial properties of flavonoids. In Dietary Phytochemicals and Microbes, AK Patra (ed.), Springer Netherlands. pp. 33-91.
Tiwari P, Kumar B, Kumar M, Kaur M, Debnath J, Sharma P (2011). Comparative anthelmintic activity of aqueous and ethanolic stem extract of *Tinospora cordifolia*. Int. J. Drug Dev. Res. 3(1):70-83.
Trease GE, Evans WC (2002). Pharmacognosy 12th Edition. Bailliere Tindal, London P 622.
Umberto Q (2000). CRC World Dictionary of Plant Names III: M-Q CRC Press. P 1730.