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Cysteine-stabilised peptide extract of *Morinda lucida* (Benth) leaf exhibits antimalarial activity and augments antioxidant defense system in *P. berghei*-infected mice

Joseph O. Adebayo[a,*], Kayode E. Adewole[a], Antoniana U. Krettli[b]

[a] Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria
[b] Laboratorio de Malaria, Centro de Pesquisas Rene Rachou, FIOCRUZ, Belo Horizonte, MG, Brazil

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ABSTRACT

Ethnopharmacological relevance: Cysteine-stabilised peptides (CSP) are majorly explored for their bioactivities with applications in medicine and agriculture. *Morinda lucida* leaf is used indigenously for the treatment of malaria; it also contains CSP but the role of CSP in the antimalarial activity of the leaf has not been evaluated.
Aim of the study: This study was therefore performed to evaluate the antimalarial activity of partially purified cysteine-stabilised peptide extract (PPCPE) of *Morinda lucida* leaf and its possible augmentation of the antioxidant systems of liver and erythrocytes in murine malaria.
Materials and methods: PPCPE was prepared from *Morinda lucida* leaf. The activity of PPCPE was evaluated *in vitro* against *Plasmodium falciparum* W2 and its cytotoxicity against a BGM kidney cell line. PPCPE was also evaluated for its antimalarial activity and its effects on selected liver and erythrocyte antioxidant parameters in *P. berghei* NK65-infected mice.
Results: PPCPE was not active against *P. falciparum* W2 (IC_{50}: > 50 μg/ml) neither was it cytotoxic (MLD_{50}: > 1000 μg/ml). However, PPCPE was active against *P. berghei* NK65 *in vivo*, causing 51.52% reduction in parasitaemia at 31.25 mg/Kg body weight on day 4 post-inoculation. PPCPE significantly reduced ($P < 0.05$) malondialdehyde concentrations in the liver and erythrocyte at higher doses compared to untreated controls. PPCPE increased glutathione concentration and activities of glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase in a dose-dependent manner, which was significant ($P < 0.05$) at higher doses compared to the untreated controls.
Conclusion: The results suggest that PPCPE may require bioactivation *in vivo* in order to exert its antimalarial effect and that PPCPE may augment the antioxidant defense system to alleviate the reactive oxygen species-mediated complications of malaria.

1. Introduction

Malaria is an infectious disease associated with considerable morbidity, mortality and significant social and economic impact on developing societies. With approximately 24 million clinical cases annually, it remains the leading cause of death due to parasitic diseases, with an estimated 438,000 deaths yearly, primarily in African children (WHO, 2015). *Plasmodium falciparum*, the most dangerous causative agent is becoming increasingly resistant to standard antimalarial drugs (Tinto et al., 2006; White, 2010). New antimalarials with novel mechanisms of action are urgently needed (Muregi et al., 2003). In Africa, a large percentage of the population rely on herbal medicine for the treatment of one ailment or the other (Willcox and Bodeker, 2004), as such a large number of medicinal plants are employed in the treatment of malaria. Some of these medicinal plants have been reported to express small cysteine-stabilised linear or cyclic peptides which were originally meant to serve as innate defense against pathogens. Some of these peptides have been reported to possess pharmacological activities such as antimicrobial, antioxidant, haemolytic, anti-cancer, insecticidal, nematocidal and immunomodulatory activities (Gründemann et al., 2013).

First discovered in the *Rubiaceae* plant, *Oldenlandia affinis,* cysteine-stabilised peptides (CSP) have been isolated from other plant families such as *Apocynaceae, Violaceae*, *Fabaceae* and *Solanaceae* (Koehbach et al., 2013). CSP distribution in plants is a subject of interest to scientist as information about the individual peptide content

* Corresponding author.
E-mail address: topebayo2002@yahoo.com (J.O. Adebayo).

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Table 1
Phytochemical constituents of crude peptide extract and partially cysteine-stabilised peptide extract of *Morinda lucida* leaf.

Phytochemicals	Crude peptide extract	Cysteine-stabilised peptide extract
Alkaloids	+	–
Anthraquinones	–	–
Flavonoids	+	–
Glycosides	–	–
Phenolics	+	+
Phlobatamins	+	–
Saponins	+	+
Steroids	+	+
Tannins	–	–

+ - Present; - - Absent.

of a single species is scanty because of the cumbersome analytical processes involved in their identification (Gründemann et al., 2013). Members of the *Rubiaceae* plant family are believed to be a rich source of CSP, one example being *Morinda lucida* (ML) Benth, commonly called Brimstone tree. It is a rainforest tree valued for its therapeutic properties in Nigeria and West Africa. *Morinda lucida* has been used for the treatment of diabetes (Burkill, 1985) and malaria (Makinde and Obih, 1985). It is also used as febrifuge, to facilitate childbirth (Elias et al., 2007), as a memory enhancer (Elufioye et al., 2013), and anticancer agent (Sowemimo et al., 2007). Till date, the contribution of CSP to the reported antimalarial activity of *Morinda lucida* leaf has not been evaluated. In a bid to get new candidates for rational antimalarial drug design, this study was carried out to evaluate partially purified cysteine-stabilised peptide extract (PPCPE) of *Morinda lucida* leaf for its antimalarial activity and its ability to augment the antioxidant system in *Plasmodium berghei*-infected mice.

2. Materials

2.1. Chemicals

Acetonitrile, trifluoroacetic acid, haemin, Giemsa stain, chloroquine diphosphate, 2, 2-diphenyl-1-picrylhydrazyl, reduced glutathione, glutathione, sodium acetate, acetic acid, dimethyl sulphoxide, thiobarbituric acid, trichloroacetic acid, epinephrine, alpha Cyano-4-hydroxycinnamic acid, Tris buffer, EDTA, saponin, sorbitol and Triton X-100 were purchased from Sigma Chemical Company, St. Louis, Mo, USA. Ascorbic acid, sodium nitroprusside, sulphanilamide, naphthylethylenediamine dihydrochloride, phosphoric acid, potassium ferricyanide, trichloroacetic acid, iron (III) chloride, tetraoxosulphate (VI) acid, sodium trioxo phosphate (III), ammonium molybdate, sucrose, sodium citrate, Dichloromethane, methanol, sodium hydroxide, sulphosalicylic acid, sodium azide, Formaldehyde, $CaCl_2$, $MgCl_2$, butylated hydroxyl toluene (BHT), KH_2PO_4, KOH, $ZnSO_4$ and sodium nitrite were purchased from Merck Pharmaceutical Company, Darmstadt Germany, C_{18}-reversed phase silica gel used was a product of Phenomenex, Aschaffenburg Germany.

Table 3
Parasitaemia in *P. berghei* NK65-infected Mice Treated with Crude Peptide Extract of *Morinda lucida* Leaf.

Dose (mg/kg body weight)	Parasitemia,% (% Reduction)		
	4°	6°	8°
Distilled water	0.16	1.75	4.05
62.5	0.13 (18.8)	1.17 (31.2)	1.52 (62.5)
125	0.12 (25.0)	0.61 (64.1)	1.33 (67.2)
250	0.14 (12.5)	0.32 (81.2)	0.81 (80.0)
500	0.05 (68.8)	0.52 (69.4)	1.09 (73.1)
Chloroquine (20)	0.05 (68.8)	0.59 (65.3)	1.16 (71.4)

Values are means (n = 5). ° Day post-inoculation.

Table 4
Parasitaemia in *P. berghei* NK65-infected mice treated with partially purified cysteine-stabilized Peptide Extract of *Morinda lucida* leaf.

Dose (mg/kg body weight)	Parasitemia,% (% Reduction)		
	4°	6°	8°
5% DMSO	0.99	2.20	3.97
15.62	0.75 (24.24)	1.18 (46.36)	1.21 (69.52)
31.25	0.48 (51.52)	0.83 (62.27)	0.87 (78.09)
62.50	0.52 (47.47)	0.66 (70.00)	0.83 (79.09)
125	0.24 (75.76)	0.53 (75.91)	0.56 (85.89)
Chloroquine (20)	0.22 (77.78)	0.28 (87.27)	0.45 (88.66)

Values are means (n = 5). ° Days after inoculation.

Table 5
Percentage Inhibition of ß-haematin formation by partially purified cysteine-stabilized peptide extract of *Morinda lucida* leaf.

Concentrations of compound/extract	% inhibition
CQ (mM)	
1	78.33 ± 0.03[a]
2	85.16 ± 0.18 [a]
4	85.76 ± 0.20 [a]
PPCPE(mg/ml)	
1	18.49 ± 0.76[b]
2	37.52 ± 0.93 [b]
4	38.53 ± 0.22 [b]

CQ, chloroquine; PPCPE, Partially purified cysteine-stabilized peptide extract. Values are means ± SEM (n = 3). Values in the same column with different superscripts are significantly different ($P < 0.05$).

2.2. Plant material

Morinda lucida (Benth) leaves were collected at Ibadan, Oyo State, Nigeria, on 22nd September 2014, and were identified and authenticated at the Forest Research Institute of Nigeria, Ibadan, Nigeria (Voucher number - FHI: 110187).

Table 2
Antiplasmodial activity and cytotoxicity of crude peptide and partially purified cysteine-stabilized peptide extracts of *Morinda lucida* leaf.

Extract	Activity against *P. falciparum* (clone W2) IC_{50} (µg/ml)			Cytotoxicity against BGM kidney cell line MLD_{50} (µg/ml)			Selectivity Index (SI)	Remark
	Exp. 1	Exp. 2	Mean ± SD	Exp. 1	Exp. 2	Mean ± SD	MDL_{50} / IC_{50}	
CPE	49.8	43	46 ± 5	> 1000	> 1000	> 1000 ± 0	> 20	Inactive
PPCPE	> 50	> 50	> 50 ± 0	379	225	302 ± 109	< 6	Inactive
Chloroquine	0.15	0.13	0.14 ± 0.01	198	112	155 ± 61	1107	Active

Each experiment was run in triplicate. CPE- Crude peptide extract of *Morinda lucida* leaf; PPCPE – Partially purified Cysteine-stabilized peptide Extract; IC_{50}- Median inhibitory concentration; MLD_{50}: Median lethal dose; BGM: Blue-Green Monkey.