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In vitro antimalarial activity of methanol extract and fractions of whole plant of *Centaurea perrottetii* D.C

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Research Article

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ABSTRACT

Malaria is a major public health problem affecting not less than 40 % of the world's population. The whole plant of *Centaurea perrottetii* DC is used in traditional medicine as anti-malarial, anti-gonorrhea and anti-syphilis agent. The aim of this study is to evaluate the in vitro antimalarial effect of C. *perrottetii* using β – hematin formation assay. The powdered whole herb (2.1 kg) of the plant was extracted with 90 % methanol and the resulting crude methanol extract (CME) was partitioned into n-hexane, chloroform, ethylacetate, n-butanol and residual aqueous fractions. The CME and its fractions exhibited significant antimalarial activity; the highest percentage inhibition of the β B hematin formation exhibited by the extract and fractions ranges from 98.66 – 99.64 % at 0.008 mg/mL which was higher than that exhibited by the standard drug, CQ (97.75 %). The IC50 values of the extract ranges from 0.0112 - 0.0114 mg/mL while CQ had 0.0114 mg/mL. The whole plant of *C. perrottetii* has demonstrated good antimalarial activity, thereby validating the ethnobotanical claim of the use of the plant in treatment of malaria.

INTRODUCTION

Studies have revealed that over the years, natural products, especially from plant origin, are the mainstay of traditional medicine because of the prominent role they have played in providing the best known antimalarial agents [1]. Malaria is a major public health problem affecting not less than 40 % of the world's population [2]. In 2018, an estimated 228 million malaria cases were reported worldwide, with about 405,000 deaths (children under five years accounted for 67 % of the total death worldwide) [3]. However, it was reported that out of 11 million pregnant women exposed to malaria infections in 2018, in 2019 16 % of them gave birth to children with low birth weight [3]. The disease poses share impediment to social and economic development through a variety of ways including school and work absenteeism, high treatment expenses decreased productivity in family. About 70 % of Nigerians are poor and majority live in rural areas. The dare economic situation in Nigeria and many other under-developed countries necessarily means that majority of the populace cannot afford the current artemisinin-based combination therapy (ACT), where available [4]. Drug-resistance strains of P. falciparum have been reported since 1960, especially to chloroquine, this has made the treatment of malaria somehow problematic in almost all malaria endemic regions [5]. The current limitations of vaccine and vector control, as well as the increasing resistance of malaria parasites to existing drugs, highlight the continuous need for new anti-malarial agents. Continuous development of anti-malarial drugs from medicinal plants is necessarily a very appealing option. Research for alternative, new, safe and effective anti-malarial agents among plants used in traditional medicines is a viable endeavor.

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The plant, *Centaurea perrottetii* has been reportedly used in ethno-medicine for the treatment of fevers and malaria [6]. Literature search to the best of our knowledge revealed that no work has been done on the evaluation of anti-malarial activity of the whole plant of Centaurea perrotteti.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material

Fresh whole plant of *Centaurea perrottetii* was collected in November, 2014 from Illela Local Government Area, Sokoto State, Nigeria and was identified and authenticated by a taxonomist, Mal. M. A. Salihu of Botany Unit, Usmanu Danfodiyo University, Sokoto. Voucher samples (voucher number UDUH/ANS/0034) were prepared and deposited for future reference. The whole plant was shade dried, pulverized, labelled and stored at room temperature for future use.

Extraction Procedures

The powdered whole plant (2.1 kg) was extracted with 90 % methanol using maceration method for 7 days. The filtered extract was concentrated in vacuo using rotary evaporator at 40°C to yield a dark green residue, subsequently referred to as the crude methanol extract, CME.

Some part of the CME (120 g) was suspended in distilled water and then filtered. The water soluble portion was partitioned successively with n-hexane (1.5 L), chloroform (500 mL), ethylacetate (1 L) and n-butanol (1.5 L) to obtain hexane (HF), chloroform (CF), ethylacetate (EF), n-butanol (BF) and the residual aqueous fractions (AQF), respectively.

Phytochemical screening

Preliminary Phytochemical tests were carried out on the crude methanol extract and its various fractions to identify the phytochemical constituents present viz. alkaloids, steroids, terpenoids, anthraquinone glycosides, flavonoids, tannins and phenolic compounds, saponins and carbohydrates using standard procedures [7, 8, 9].

Antimalarial β B hematin assay

Inhibition of β-hematin synthesis was conducted according to the method described by Baelmans et al. [10]; a solution of hematin porcine in DMSO (Dimethylsulfoxide) (50 µL, 5.2 mg/mL) was distributed in 96-well micro-plates. Different concentrations of all the fractions, was dissolved in DMSO and added in triplicates in the test wells (50 µL) final concentrations was between 0.002 and 0.032 mg/mL. Controls contained 5.2mg/mL DMSO only (negative) and chloroquine (5 mg/mL) (positive). β-Hematin synthesis was initiated by the addition of 100 µL of 0.2 M sodium acetate buffer at pH 4.4. Plates were incubated at 37°C for 48 h. The incubated plates were centrifuged at 4000 rpm for 15 min. After discarding the supernatant, the pellet was washed three times with DMSO (200 L) and finally dissolved in 200 L, 0.2N NaOH solution. The solubilized aggregates were further diluted at 1:2 with NaOH solution (0.1 N) and absorbance recorded at 405 nm (Microplate Reader, BIORAD-550). The results were expressed as percentage inhibition of β-hematin synthesis compared to negative control. The effective concentrations of sample required to inhibit the β-hematin synthesis by 50 % (IC50 value) [11].

Statistical analysis

All experiments were performed in triplicate and presented as the Mean ∀ SD. Data were analyzed by Microsoft Excel 2010. The IC50 and IC90 values were calculated by nonlinear regression analysis.

RESULTS

The crude methanol extract of *C. perrottetii* and its various fractions contain different secondary metabolites such as alkaloid, carbohydrate, flavonoid, cardiac glycoside, saponin, tannin, steroid and terpene (Table 1).

The crude methanol extract of *C. perrottetii* and its various fractions exhibited different percentage inhibition of β-hematin formation of 0.008 mg/mL, the effect was higher than that observed for standard drug, CQ (Figure 1).

The AQF had the highest % inhibition (99.64 %) of β-hematin formation while n – butanol fraction had the least (98.68 %) % inhibition (Figure 1).

The IC50 values of the extract and fractions of *C. perrottetii* ranges from 0.0112 – 0.0114 mg/Ml (Table 2).Key: CME= Crude

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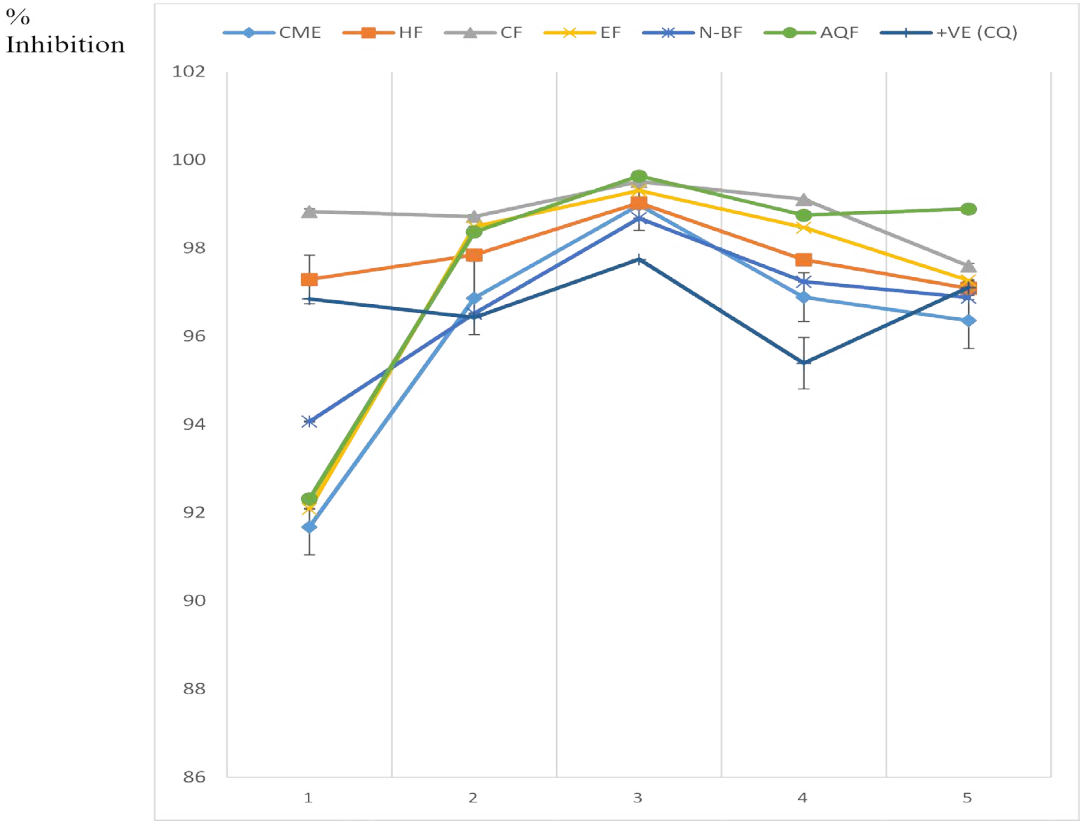
Table 1: Phytochemical constituent of crude methanol extract and the various fractions of C. perrottetii

Constituents	Observation	Inference					
		CME	n-HF	CF	EF	nBF	AQF
Saponins	Frothing persist for 15mins	+	-	-	+	+	+
Alkaloids	White-cream ppt	+	-	+	+	+	-
	Orange ppt						
	Reddish-brown ppt						
Flavonoids	Green or violet ppt Orange-red ppt	+	-	-	+	+	+
Tannins	Cream ppt	+	-	-	+	+	+
Steroids and Terpenes	Blue-green color at interphase Reddish color	+	+	+	-	-	-
Anthraquinones	Pink or violet	-	-	-	-	-	-
Carbohydrates	Reddish ring Red	+	+	+	-	-	+
Phenols	Bluish black color	+	+	-	+	+	+
Glycosides	Pink-blood red ppt	+	-	-	+	+	+



Extracts	IC_{50} (mg/mL)
CME	0.0114
HF	0.0113
CF	0.0112
EF	0.0113
n-BF	0.0113
AQF	0.0112
+VE (CQ)	0.0114

Methanol Extract, HF= Hexane Fraction, CF = Chloroform fraction, EF= Ethyl-acetate fraction, n-BF= n-Butanol Fraction, AF = Aqueous Fraction, CQ = Chloroquine phosphate, + VE = positive control.

DISCUSSION

The crude methanol extract (CME) and the positive control (CQ) exhibited the same IC50 (0.0114 mg/mL), n-hexane (HF), ethylacetate (EF) and n-butanol (n-BF) fractions exhibited the same IC50 (0.0113 mg/mL) while chloroform (CF) and residual aqueous (AQF) fractions exhibited the same IC50 (0.0112 mg/mL) IC50 at different H4concentration (Table 2).

Due to continuous emergence of resistant strains of Plasmodium faalciparium, there is need for continuous search of new antimalarial agents. Sequel to that, methanol extract of *C. perrottetii* and its fractions were screened for their ability to inhibit β-hematin formation. Formation of malaria pigment or hemozoin is the major route of heme detoxification in the malarial parasite [12]. Malaria parasites proteases break down the hemoglobin of the host for survival [13]. By this act, these parasites (plasmodium species), despite the absence of heme oxygenases, are able to detoxify heme which is harmful to them, and convert it to hemozoin (β-hematin is the homologue of hemozoin), also known as malaria pigment [13]. This implies that, substances or compounds which are able to inhibit the synthesis of this malarial pigment (β-hematin or hemozoin) and the degradation of haemoglobin might be useful anti-malarial agents. Moreover, inhibition of heme biocrystalization has been used as one of the major target for drug discovery in the fight against malaria [14, 15].

Inhibition of the conversion or polymerization of heme to β-hematin (hemazoin or malaria pigment) will lead to accumulation of heme in the parasite food vacuole thereby causing the death of the parasites [12, 16,17]. The .in vitro anti-malarial study revealed that 50 % inhibitory concentration (IC50) of the crude methanol extract was comparable to that of the standard drug, chloroquine, i. e 0.0114 mg/mL while the n-hexane, ethyl-acetate and n-butanol fractions revealed similar IC50 of 0.0113 mg/mL (11.3 µg/mL). The observed IC50 for chloroform and residual aqueous fractions was 0.0112 mg/mL (11.2 µg/mL). The ability of the extract to inhibit the formation of β-hematin might be due to the presence of some bioactive secondary metabolites that were detected in the extract and the fractions, thus the antimalarial effect of flavonoids, saponins alkaloids and steroids have been reported [18, 19].

Conflict of interest

We declare that we have no conflict of interest

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