DOI: 10.55522/jmpas.V11I2.3160 ISSN NO. 2320–7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

In vivo and in vitro antiplasmodial activity of extracts, fractions and an isolated compound from silk of zea mays (corn silk)

Nestor Tordzagla[1]*, Isaac Ayensu[2], Michael Fokuo Ofori[3]

[1] Department of Pharmaceutical Chemistry, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana.
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[3] Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Legon, Accra.

ABSTRACT

This study has investigated antiplasmodial activity of extracts, fractions and an isolated compound from corn silk using both *in vivo* and *in vitro* assays. Also, safety studies were carried out on the extract. Pharmacologically, both aqueous and methanolic extracts demonstrated remarkable *in vivo* antiplasmodial activity of 78.9 ± 1.59 % and 77.3 ± 0.92 % reduction in parasitaemia respectively. The bioassay guided fractionation indicated that ethyl acetate fraction of the methanolic extract of corn silk exhibited highest antiplasmodial activity among the fractions investigated. Employing column chromatography and other chromatographic methods on the ethyl acetate fraction of the methanolic extract of corn silk led to isolation of norbergenin. Spectroscopic methods such as infrared, one-dimensional, two-dimensional nuclear magnetic resonance and others were used in characterization and identification of the compound. Antiplasmodial investigation of the compound using *in vitro* SYBR Green 1 fluorescent-based assay on the chloroquine-sensitive based plasmodial 3D7 strain showed that it possessed antiplasmodial activity of IC_{50} value of 15.31±2.10 µg/mL and could be linked to the bioactive compounds in ethyl acetate fraction. The results of this study have granted support to the local use of corn silk for the treatment of malaria. It is the first report of these pharmacological studies. It is also the first report of isolating norbergenin from corn silk.

Keywords: Antiplasmodial, In Vivo, In Vitro, Zea Mays, Phyto constituents.

Received - 04-03-2022, Accepted- 24-05-2022

***Correspondence:** Nestor Tordzagla ✉ ntordzagla@uds.edu.gh **Orcid Id**: https://orcid.org/0000-0002-6593-5814

Department of Pharmaceutical Chemistry, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana

INTRODUCTION

Malaria is one of the highly infectious diseases and it is endemic in 90 countries. According to the World Health Organization (WHO) report in 2018, 219 million malaria cases occurred globally and it was an increase of 2 million cases over the previous year. Nine-two percent (92 %) of the cases came from Africa and 93 % of all malaria deaths representing 435 000 deaths occurred in Africa. In Africa, Plasmodium falciparum is the malaria parasite accountable for most of the deaths and responsible for 99.7 % of the total malaria cases [1]. It is indisputable fact that malaria has engulfed African population.

In Ghana for instance, the malaria prevalent rate is very high and more pronounced immediately after the rainy season [2]. The population of Ghana stood at 28.8 million, according to the 2017 census [3], and the entire population of Ghana is at risk of malaria infection [4]. The percentage malaria cases confirmed in Ghana from 2014 to 2018 were 53.5 %, 61.7 %, 65.7 %, 79.0 % and 85.4 %, respectively. Although, malaria related deaths have declined in the country as a result of many malaria control interventions put in place by Ghana Health Service [5]. However, Plasmodium falciparum remains the deadliest species accounting for 85-90 % of all malaria infections in Ghana [2].

Drug discovery from traditional medicines has been an important component of drug development and a source of lead compounds for treatment of many diseases. Typical examples are artemisinin, quinine and their derivatives [6]. Medicinal plants have many secondary metabolites and some of these metabolites are responsible for many bioactivities [7, 8]. Corn silk has many medicinal uses and notable among them are antioxidant properties [9], anti-diabetic activity [10], and antitumor activity [11].

Traditionally, corn silk has been used as a medicine for treating hypertension, hyperglycemia, tumor and hepatitis [12]. Moreover, the dried corn silk is used in Ghana as a traditional medicine for treating malaria and other infectious diseases.

The antiplasmodial activity of corn silk has not been thoroughly investigated. The phytochemical constituents responsible for pharmacological activities and acute toxicity study of this plant

have not been investigated despite its continuous use in traditional medicine to treat malaria. This research work remains an important factor to thoroughly study this part of the medicinal plant to scientifically validate its use and evaluate its toxicity. Most importantly, isolating and structurally characterizing the active constituents present in corn silk.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Corn silk which is also known as stigmas of Zea mays was collected from Ejisu in the Ashanti Region, in June, 2015 then identified and authenticated by Mr. Clifford Osafo Asare, the horticulturist with the Department of Herbal Medicine, KNUST, and the specimen placed in the herbarium of the Department with a voucher number KNUST/HM/2016/001.

Preparation of Plant Samples and Extraction

A 950 g of milled corn silk was boiled in 3.9 L of distilled water for 15 minutes and filtered when the solution was hot using white cloth gauze. The mac obtained was dried and re-extracted with distilled water for one more time and filtrates were concentrated using freeze dryer (Heto power dry LL300 with the pump Edwards 113GC 11B T_4). The lyophilized extract was used in the in vivo antiplasmodial assays.

In addition, 2.28 kg of another powered corn silk was extracted with 7.3 L of methanol for 72 hours using cold maceration method. After the three days it was decanted and filtered using Whatman filter paper 1 and the mac was also re-extracted with methanol and filtered. The filtrates were concentrated to dryness on a Buchi rotavapour R-210 with the vacuum pump V-710V on Buchi water bath B-491 at 40 ^oC. About 5.0 g of the extract obtained was used for initial in vivo antiplasmodial assays involving 4-day suppressive test and curative test.

Furthermore, 15 g of the methanolic extract was dissolved in a separatory funnel containing 500 ml of distilled water and washed sequentially with 300 ml x 3 portions each of petroleum ether, chloroform and ethyl acetate respectively. The various fractions were also concentrated to dryness on Buchi rotary evaporator except the aqueous fraction, which was freeze dried and these extracts were also screened for in vivo antiplasmodial activity. The antiplasmodial screening of all the extracts was done after phytochemical screening and acute toxicity studies were done on the extracts.

Phytochemical Screening

The methanolic and aqueous extracts were screened for the existence of the phytochemicals such as flavonoids, alkanoids, saponins, tannins, reducing sugars, coumarins, triterpenes, sterols, cyanogenetic glycosides and cardiac glycosides by employing the standard methods as described by Evans [13].

Ethical Consideration

The study was approved and carried out under the surveillance of the Centre for Plant Medicine Research ethical committee, Mampong-Akuapem of Ghana; in the year, 2015 with the certificate number CPMR-ET/M.02 2015 and it was in agreement with the Guide for Care and Use of Laboratory Animals [14].

Acute Oral Toxicity Test

The test was done in accordance with the Organization for Economic Cooperation and Development (OECD) 425 guideline for testing chemicals [15]. The pathogen free Imprinted Control Region (ICR) mice of both sexes in groups of six were fasted overnight and orally administered with a single dose of 2000 and 5000 mg /kg body weight of each extract or 10 mL/kg normal saline 0.9 % for normal control group. The experimental animals were observed closely for weight loss, diarrhea, decreased in movement, skin fur position, lethargy, salivation or death at 0, 30, 60, 120 and 200 min, 24 h and 14 days after administration of the extracts.

In Vivo Antiplasmodial Assay

Malaria parasite strain

The malaria parasite strain, Plasmodium berghei ANKA (NK 65) was used in the in vivo study and was donated by the Department of Immunology of the Noguchi Memorial Institute for Medical Research, University of Ghana. The parasites were kept alive throughout the study by continuous intraperitoneal passage of the parasites in mice which served as the donor mice [16]. Prior to the actual assay, the donor mice were kept and observed to show malaria symptoms similar to malaria symptoms in humans [17].

Animals used for in vivo assay

The pathogen free Imprinted Control Region (ICR) mice were used in this study. They were obtained from the Animal House Department of Centre for Plant Medicine Research (CPMR), Mampong- Akuapem, Ghana. The mice used in this research work were made up of either sex with weight range of 25-33 g and were between 8-12 weeks old. For suppressive test and curative test, five mice (N=5) were used in each group.

Inoculation of parasite in mice and determination of parasitaemia

Inoculation of Plasmodium berghei in donor mice as well as the experimental mice was done by following standard method. The total number of erythrocytes/ mL of the blood of the infected mouse were first estimated using the Haemocytometer with cover glass. After that, the total concentration of 5.0×10^7 per mL was prepared by diluting the blood collected from the infected mouse with phosphate buffered saline in a heparinized tube. The final inoculation concentration containing 1.0×10^7 of P. berghei parasitized red blood cells in 0.2 mL was prepared. Each mouse was then intraperitoneally inoculated on day 0 with 0.2 mL with the final inoculation concentration [18].

The percentage parasitaemia was determined using equation 1 by counting infected erythrocytes in five fields, divided by the total erythrocytes in the five fields then multiplied by hundred [19].

$$\text{Percentage parasitaemia} = \frac{\text{number of infected red blood cells/field}}{\text{total number of red blood cells/field}} \times 100\%$$

…Equation 1

Four-day suppressive test

The schizonticidal activity of the two extracts was assessed using Plasmodium berghei infected mice in a four-day suppressive test using a standard protocol [18]. Three different doses of each extract, 125 mg/kg body weight, 250 mg/kg body weight and 500 mg/kg body weight were used. The mice in negative control group were given 0.2 mL of distilled water while those in positive control group received arthemether lumefantrine 4 mg/kg body weight. The treatment was done once daily and the first day of inoculation was named D_0. Treatment with the test drugs and standard drug commenced 3 hours after inoculation and continued daily for four days from day 0 to day 3. At the end of the four-day treatment, thin blood film was prepared from the tail of each mouse and average percentage parasitaemia and suppression were determined [18, 20].

Moreover, the average survival duration of experimental mice for the various groups was evaluated by computing the mean survival duration for a period of 30 days. The results were statistically analysed using One-Way ANOVA from GraphPad Prism v 5 at 95 % confidence interval.

Curative test

This test was used to evaluate the curative capability of both extracts as well as their fractions and was done in accordance with the standard method of curative in vivo assay [21]. On the first day (Do), the experimental mice were infected as in four-day suppressive test. However, treatment commenced 72 hours after infection and continued once daily for five days. Three different doses of 125 mg/kg, 250 mg/kg and 500 mg/kg body weight were used as test drugs. Arthemether-lumefantrine 4 mg/kg body weight was used as a standard drug and served as the positive control. The negative control groups were two, negative control for aqueous extracts assays and that of organic fractions assays, for the first group, 0.2 mL each of distilled water was given to each mouse and for the later, 5 % dimethyl sulfoxide (DMSO) in normal saline was given to each mouse. Parasitaemia was monitored daily using microscopic examination of Giemsa-stained thin blood smears prepared from the tail of each mouse daily for five days. Percentage parasitaemia and parasitaemia suppression were parameters calculated using equation 2.

Mean of percentage parasitaemia suppression

$$= \left[\frac{A-B}{A}\right] \times 100 \ldots\ldots \text{ Equation 2}$$

Where A is the mean of percentage parasitaemia of the negative control group (untreated group) and B is the mean of percentage parasitaemia of the various treated groups.

Results were analysed statistically by using Two-Way ANOVA followed with Tukey and Bonferroni post- tests from GraphPad Prism v 5 at 95 % confidence interval.

Isolation and Identification of the Bioactive Compound

The pharmacological assays have proved that ethyl acetate fraction demonstrated the highest in vivo antiplasmodial activity among the set of fractions investigated from corn silk, therefore, it was selected for column chromatography, leading to isolation, characterization and identification of the bioactive constituent.

Silica gel gravity column chromatography and thin layer chromatography column were chromatographic techniques employed in the isolation of the bioactive constituent from the fraction. Column chromatography (LC) was performed using silica gel 70:230 mesh size (Merck, Germany) and silica gel 90 C_{18} reversed phase ((Sigma Aldrich, U.S.A.) for purification of the compound.

Characterization and identification of the compound was carried out using a Joel ECA, 500 MHz FT Nuclear Magnetic Resonance (NMR) Spectrometer (NM 103508-10, Japan) incorporating a NM-50TH5AT/FG2 probe for running 1D, 2D NMR and DEPT-135 spectra, Liquid Chromatography-Mass Spectrometry (LC-MS), LC infinity 1290 with mass spectrometer 6420 QQQ ms (USA) for running mass spectrum of the compound to confirm the identity of the molecule, infrared (PerkinElmer ATR-FTIR spectrometer, USA) was run to know the functional groups present in the compound, and melting point determination (Stuart, UK./R000105350 melting point apparatus).The spectroscopic data obtained was compared with reported data.

In vitro Antiplasmodial Assay

The preparation of the isolated compound and the reference drug as well as the entire in vitro assay using the fluorescence-based SYBR Green 1 method followed the test protocol described in the literature [22].

IC_{50} Determination by SYBR Green assay

The parasitized plates were thawed for about 2 h at room temperature and 100 µL of prepared SYBR Green 1 buffer was added to each parasitized plate and the plates were covered with aluminum foil and incubated at room temperature for 2 h. A microtitre plate reader spectrophotometer (Infinite M200, Tecan, Austria) was used to read the plates by fluorescence detection of intensity measured at wavelength of 485 nm excitation and 530 nm emission. The drug concentration that inhibits the growth of 50 % Plasmodium falciparum cells (IC_{50}) was estimated by using GraphPad Prism 5,

software, fitting a log dose versus response curve with the linear regression option.

Data Analysis

The in vivo and in vitro results were analysed statistically by using GraphPad Prism Windows version 5 (GraphPad Software, San Diego, CA, USA). The results of four-day suppressive tests were analysed using One-Way ANOVA at 95 % confidence interval while Two-Way ANOVA followed by Bonferroni post-test was used to analyse results obtained from the curative tests. Also, IC_{50} values of the isolates and the standard drug were determined using the GraphPad Prism.

RESULTS AND DISCUSSION

Phytochemical Screening

The preliminary phytochemical screening carried out on aqueous and methanolic extracts from the silk of Zea mays showed the presence of important secondary plant metabolites among them were; flavonoids, alkanoids, saponins, tannins, reducing sugars, coumarins, triterpenes, sterols and cardiac glycosides. However, cyanogenetic glycosides were absent. The confirmation of these secondary metabolites has set a good platform to investigate the bioactive effects of corn silk.

Acute Oral Toxicity Test

The preliminary acute oral toxicity study to check behavioural toxicity signs and also mortality of the experimental mice has shown that the two extracts from corn silk have their LD_{50} values greater than 5000 mg/kg, hence, they have high degree of safety (OECD, 2008). According to the chemical labeling and classification of acute systemic toxicity recommended by OECD, a crude extract of LD_{50} > 5000 mg/kg has its lowest toxicity.

Four-Day Suppressive Test

The suppressive test results showed that the extracts from corn silk and the standard drug (A/L) had reduced schizonts maturation of Plasmodium berghei in infected mice significantly. The treatment also resulted in relative increase in survival days of the experimental mice as compared to the negative control groups. Also, the average survival days recorded for the mice that were treated with the standard drug at 4 mg/kg was greater than 30.0±0.00 days and its percentage parasitaemia suppression was 99.8±0.11. Moreover, the percentage parasitaemia suppressions for the extracts at 250 mg/kg and 125 mg/kg were not significantly different from 500 mg/kg at P < 0.001, (Table 1).

Table 1: Four-day suppressive test of extracts from corn silk

Dose (mg/kg)	CSA		CSM	
	% Suppression	Survival Time (days)	% Suppression	Survival Time (days)
500	78.9±1.59***	22±1.58	77.3±0.92***	20±1.26
250	72.7±1.14***	21±0.98	74.2±1.02***	20±1.95
125	69.8±1.44***	19±1.26	70.1±1.57***	17±1.82

Values are expressed as mean± S.E.M. (n = 5). Negative control group recorded no percentage suppression. Suppressive test against schizonts maturation of Plasmodium berghei in infected mice using aqueous extract of corn silk (CSA), methanolic extract of corn silk (CSM). One-Way ANOVA from GraphPad Prism v 5 at 95 % confidence interval. $***P < 0.001$ denotes significance level when compared to negative control group (NC).

According to literature, plant extracts or drugs with percentage plasmodial suppression ≥ 40 % in vivo by day three and onwards are deemed active [20]. It therefore means that the extracts and the standard drug were deemed active against Plasmodium berghei strain used in this study.

Curative Activities of the Extracts, Fractions and Reference Drug

The curative test was used to evaluate the curative capability of extracts, fractions and the reference drugs on established infections. All the extracts, fractions and the reference drug showed no significant (P > 0.05) reduction in the parasitaemia on day one after treatment. However, from day two after treatment, significant decrease in the parasitaemia was observed with the highest reduction on the last day after treatment in most cases (Tables 2, 3 and 4). The appreciable clearance of the parasites from day two onwards may be attributed to high concentration of bioactive phytochemicals in the blood and other parts of the body as a result of repeated administration. As a result of that, the bioactive phytochemicals gained access into the parasites and exhibited the therapeutic effects.

Table 2: Curative effects of various extracts from corn silk at 500 mg/kg body weight and artemether-lumefantrine (4 mg/kg body weight)

Day after treatment	NC	A/L	CSA	CSM
	% Suppr	% Suppr	% Suppr	% Suppr
Day 1	-	17.7 ± 1.00	11.3 ± 1.69	9.9 ± 0.68
Day 2	-	38.6 ± 0.70***	24.9 ± 1.54**	22.4 ± 1.11*
Day 3	-	86.7 ± 1.52***	53.4 ± 1.96***	46.5 ± 1.73***
Day 4	-	100 ± 0.00***	61.6 ± 1.30***	58.2 ± 1.15***
Day 5	-	100 ± 0.00***	71.2 ± 1.59***	69.4 ± 1.89***

Values are expressed as mean ± S.E.M. (n=5). Suppr = Suppression. - = No percentage suppression, A/L=Artemether-lumefantrine. The treatment started on day 3 post infection, up to day-7. Daily estimation of parasitaemia for the experimental groups was done. Each point represents Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ denote significance level when compared to NC group.

Table 3: Curative effects of various extracts of corn silk at 250 mg/kg body weight and artemether-lumefantrine (4 mg/kg body weight)

Day after treatment	NC	A/L	CSA	CSM
	% Suppr	% Suppr	% Suppr	% Suppr
Day 1	-	15.3±0.93	10.4±1.04	9.5±1.26
Day 2	-	37.8±2.06***	21.3±1.55*	21.5±1.59*
Day 3	-	88.5±1.34***	45.7±1.13***	43.8±1.06***
Day 4	-	100.0±0.00***	58.6±1.37***	55.5±2.43***
Day 5	-	100.0±0.00***	66.8±1.00***	64.3±1.56***

Values are expressed as mean ± S.E.M. (n=5). Suppr = Suppression. - = No percentage suppression.The treatment started on

day 3 post infection, up to day-7. Daily estimation of parasitaemia for the experimental groups was done. Each point represents Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ denote significance level when compared to NC group

Table 4: Curative effects of various extracts of corn silk at 125 mg/kg body weight and artemether-lumefantrine (4 mg/kg body weight)

Day after treatment	NC % Suppr	A/L % Suppr	CSA % Suppr	CSM % Suppr
Day 1	-	17.2±0.60	6.5±1.38	7.2±2.10
Day 2	-	35.9±1.02***	17.8±2.14*	19.6±2.03*
Day 3	-	85.6±0.11***	41.3±1.50***	40.9±1.67***
Day 4	-	99.8±0.24***	49.8±1.77***	52.4±1.99***
Day 5	-	100.0±0.00***	60.1±2.06***	61.8±2.02***

Further analysis of the various fractions from the extracts revealed that both ethyl acetate and chloroform fractions possessed significant antiplasmodial activity (Tables 5, 6 and 7). Scientifically, there was no significant difference in antiplasmodial activity between the ethyl acetate fraction (500 mg/kg body weight) and reference drug at 95 % confidence level (Table 5). It is the first report of investigating these fractions from corn silk against Plasmodium berghei infected mice. Bioassay guided isolation of phytoconstituents from the chloroform fraction as well as ethyl acetate fraction of methanolic extract of corn silk could give a lead compound.

Table 5: Curative effects of various fractions from corn silk at 500 mg/kg body weight and artemether- lumefantrine (4 mg/kg body weight)

Day after treatment	**A/L**	**EACS**	**CCS**	**PCS**	**ACS**
	% Suppr	**% Suppr**	**% Suppr**	**%Suppr**	**% Suppr**
Day 1	15.2±1.06	13.0±1.53	10.5±0.78	3.0±0.66	8.9±0.73
Day 2	34.1±1.61***	27.9±1.13***	21.9±1.01**	3.8±1.40	12.8±1.08
Day 3	80.7±1.80***	51.6±0.93***	42.4±1.42***	4.5±1.39	24.8±1.36***
Day 4	98.6±0.22***	66.2±0.97***	51.7±1.56***	5.0±0.71	29.3±1.82***
Day 5	100±0.00***	73.5±1.41***	57.9±1.60***	4.9±1.81	32.6±1.25***

Values are expressed as mean ± S.E.M. (n = 5). Suppr = Suppression. Negative control (NC) group showed no percentage suppression. Curative effects of ethyl acetate fraction of corn silk (EACS), aqueous fraction of corn silk (ACS), chloroform fraction of corn silk (CCS), petroleum ether fraction of corn silk (PCS) and A/L on the Plasmodium berghei infection in mice. The treatment started on day 3 post infection, up to day-7. Daily estimation of parasitaemia for the experimental groups was done. Each point represents Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ denote significance level when compared to NC group.

Table 6: Curative effects of various fractions from corn silk at 250 mg/kg body weight and artemether- lumefantrine (4 mg/kg body weight)

Day after treatment	**A/L**	**EACS**	**CCS**	**PCS**	**ACS**
	% Suppr	**% Suppr**	**% Suppr**	**% Suppr**	**% Suppr**
Day 1	14.1±1.30	11.9±1.31	6.4±1.23	1.8±1.06	5.3±0.95
Day 2	36.0±1.47***	23.7±1.08**	19.2±0.94*	2.6±1.45	9.9±1.24
Day 3	79.2±1.08***	48.7±1.22***	41.5±1.59***	3.9±2.24	17.9±1.08*
Day 4	100.0±0.00***	61.3±1.14***	48.7±1.47***	4.2±1.66	21.3±1.27***
Day 5	100.0±0.00***	68.8±1.20***	52.6±1.00***	3.7±1.54	22.9±1.33***

Values are expressed as mean ± S.E.M. (n = 5). Negative control (NC) group showed no percentage suppression. Each point represents Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ denote significance level when compared to NC group.

Table 7: Curative effects of various fractions from corn silk at 125 mg/kg body weight and artemether-lumefantrine (4 mg/kg body weight)

Day afterTreatment	**A/L % Suppr**	**EACS % Suppr**	**CCS % Suppr**	**PCS % Suppr**	**ACS % Suppr**
Day 1	16.5±1.48	8.3±1.44	3.2±2.36	1.2±1.36	4.6±1.51
Day 2	35.7±0.87***	19.5±2.01*	11.6±2.15	1.9±1.57	6.2±2.40
Day 3	83.2±1.55***	42.3±1.28***	25.7±1.33***	3.1±1.99	16.7±1.17*
Day 4	99.7±0.16***	55.1±1.49***	33.1±1.82***	2.5±2.40	19.4±1.38***
Day 5	100.0±0.00***	62.7±1.16***	42.9±1.77***	2.1±1.08	18.9±2.11***

Values are expressed as mean ± S.E.M. (n=5). Negative control (NC) group showed no percentage suppression. Each point represents Mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ denote significance level when compared to NC group.

Further Phytochemical Investigations

Characterization of the compound

The compound was isolated as a white crystal. The molecular formula was assigned as $C_{13}H_{14}O_9$ based on the quasi-molecular ion peak at m/z 315.3 $[M + H]^+$ (calcd for 314.24) in the LC-MS spectrum. The LC-MS spectrum showed key fragments at m/z 337.3 $[M + Na]^+$, 297.3 $[M + H - H_2O]^+$ and 279.3 $[M+ H-2H_2O]^+$. It gave a positive test result with iron (lll) chloride solution, showing coumarin glycoside**.** The melting point was determined to be 235-237 ^{0}C and the R_f value of 0.31 (chloroform / methanol / acetic acid: 7:1:1). The FTIR (neat): Vcm^{-1} spectrum of the isolate showed a broad band at 3343 cm^{-1} and could be attributed to hydroxyl group (OH). The observation of a very intense band at 1698 cm^{-1} was as a result of C=O bond. Also, aromatic C=C was observed at 1598 cm^{-1} and a very intense band at 766 cm^{-1} is characteristic of aromatic C-H bending. The C-C vibration was recorded at 1048 cm^{-1}. A band at 1217 cm^{-1} represents C-O group of an ester.

Figure 1: Shows COSY and HMBC correlation of norbergenin

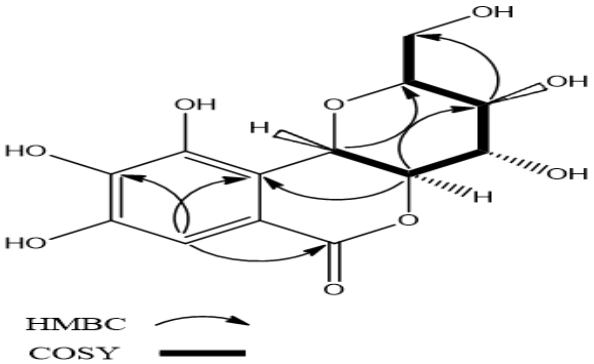
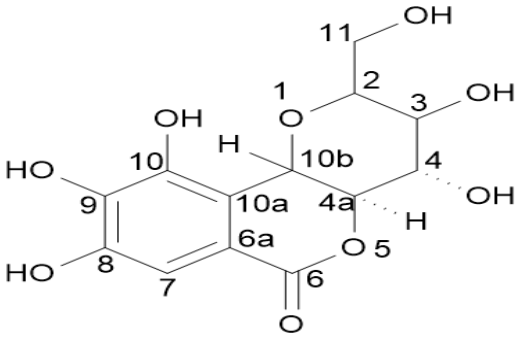


Figure 2: Structure of norbergeni



The summary of the results from ^{1}H and ^{13}C NMR are presented in Table 8. The ^{1}H NMR and ^{13}C NMR values for the protons and carbons were assigned on the basis of DEPT-135, COSY, HSQC and HMBC correlations and the chemical shift values of the compound were compared with the reported literature for norbergenin [23]. Figure 1 shows COSY and HMBC correlations of norbergenin. It is clear that the compound is identical to norbergenin (Figure 2)

Table 8: ^{1}H and ^{13}C NMR data of norbergenin in deuterated methanol

Position	$^{13}C^{b}$	$^{13}C^{*}$	Nature of carbon	$^{1}H^{a\ Multi}$. (J in Hz)	^{1}H *Multi. (J in Hz)
2	83.1	83.0	CH	3.72, m	3.68, m
3	72.1	72.0	CH	3.22, m	3.42, m
4	75.8	75.7	CH	3.86, dd (9.4, 8.9)	3.81, dd (9.2, 8.8)
4a	81.6	81.5	CH	3.93, dd (10.2, 9.0)	4.03, dd (10.4, 9.2)
6	166.6	166.6	C		
6a	117.5	117.4	C		
7	111.2	111.0	CH	6.99, s	7.08
8	147.5	147.4	C		
9	141.4	141.5	C		
10	143.8	143.8	C		
10a	114.5	114.2	C		
10b	74.5	74.4	CH	4.78, d (10.3)	4.94 d (10.4)
11	62.8	62.8	CH_2	3.59, m	3.68, m

[a]Recorded at 500 MHz; [b]Recorded at 125 MHz. *^{13}C and *^{1}H NMR (deuterated methanol) spectral data of norbergenin reported by literature [23].

In vitro Antimalarial Screening of the Isolate

The in vitro results showed that the isolate and the reference drug recorded IC_{50} values of 15.31±2.10 µg/mL and 0.04±1.60 µg/mL, respectively. It means that the phytochemical compound isolated from corn silk (norbergenin), displayed moderate antiplasmodial activity against 3D7 strain of P. falciparum in the SYBR Green 1 assay.

According to literature, the following criteria are used to consider the in vitro antiplasmodial activity of drugs: IC_{50} 1-10 µg/mL – good activity; IC_{50} 10-50 µg/mL – moderate activity; IC_{50} 50-100 µg/mL – weak activity; IC_{50} >100 µg/mL – inactive [24].

Norbergenin, 4-O-galloylnorbergenin, bergenin, and 11-O-galloylbergenin are derivatives of isocoumarins. Little is known about their antiplasmodial activities, especially of norbergenin and 4-O-galloylnorbergenin [25]. However, antiplasmodial activities of IC_{50} 6.92±0.43 and 7.85±0.61µM were reported for bergenin, and 11-O-galloylbergenin isolated from Mallotus philippensis respectively [26]. It is obvious that, the antiplasmodial activity exhibited by norbergenin isolated from the ethyl acetate fraction of methanolic extract of corn silk in this study was in line with its derivatives.

CONCLUSIONS

The finding of this study has shown that the aqueous and methanolic extracts of silk of Zea mays (corn silk) possess antiplasmodial activity. The decline in parasitaemia as well as the high survival time in the treated mice justifies the folkloric use of corn silk in the treatment of malaria. The bioassay guided fractionation showed that the ethyl acetate fraction of the methanolic extract of corn silk exhibited the highest in vivo antiplasmodial activity among the set of fractions investigated. Norbergenin was isolated from the ethyl acetate fraction. It is the first report of norbergenin being isolated from corn silk. Antiplasmodial investigation of the compound showed that it possessed antiplasmodial activity. The finding also points to the possible presence of other potentially effective antimalarial phytoconstituents in the corn silk.

Antimicrobial and anthelmintic activities of corn silk extracts and the isolate are currently being investigated.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare

ACKNOWLEDGMENT

We are very grateful to the technical staff in the Department of Immunology, Noguchi Memorial Institute for Medical Research (N.M.I.M.R), University of Ghana and Animal House Unit of the Centre for Plant Medicine Research (C.P.M.R.). Finally, we appreciate the herbalist Mr. Clifford Asare for his assistance.

REFERENCES

1. A WHO, 2018. Fact sheets: Report on infectious diseases, Geneva, Switzerland.
2. President's Malaria Initiative (PMI), 2016. Ghana: Malaria Operational Plan FY.
3. World Bank, 2017. Ghana Population Estimates and Projections.
4. WHO, 2019. World malaria report, comprehensive update on global and regional malaria data and trends.
5. President's Malaria Initiative (PMI), 2020. Ghana: Malaria Operational Plan FY.
6. Phillipson JD, Wright CW, Kirby GC, et al, 1993. Tropical plants as sources of antiprotozoal agents, In: Downum KR, Romeo JT and Stafford HA (Edition.). Phytochemical Potential of Tropical Plants. Recent Advances in Phytochemistry, Springer; Boston, MA, pp. 1-40.
7. Ayensu I, Quartey KA, 2015. Phytochemical Screening and In-vitro Antioxidant Properties of the Stem Bark of Trichilia tessemannii (Harms) (Meliaceae). WJPPS, 4(3), pp. 76-90.
8. Baidya M, Maji HS, Bhatt S, et al, 2022. In-vitro Evaluation of the Thrombolytic and Anti-inflammatory Activity of Capparis sepiaria Root Extracts. J. Med. P'ceutical Allied Sci. 11(1), pp. 4166- 4171.
9. Liu J, Wang C, Wang Z, et al, 2011. The antioxidant and free-radical scavenging activities of the extract and fraction from corn silk (Zea mays L.) and related flavone glycosides. Food Chemistry, 126(1), pp. 261-269.
10. Guo J, Liu T, Han L, Liu Y, 2009. The effects of corn silk on glycaemic metabolism Nutr Metab (Lond), 6(1), pp. 47-52.
11. Habtemariam S, 1998. Extract of corn silk (stigma of Zea mays) inhibits the tumour necrosis factor-alpha- and bacterial lipopolysaccharide-induced cell adhesion and ICAM-1 expression. Planta Med. 64(1), pp. 314-318.
12. Ma H, Gao L, 1998. Study on effect of corn silk extract (ESM) on K562 and SGS cell. J. Nanjing Univ. Traditional Chinese Med. (China), 1, pp. 28-29.
13. Evans WC, 2002. Trease and Evans Pharmacognosy, In: Saunders WB (Edition.). Trease and Evans Pharmacognosy, London; Elsevier Limited, pp. 137–49.
14. National Research Council, 2011. Guide for the care and use of laboratory animals, 8th edition. Washington (DC), US.
15. Organization of Economic Co-operation and Development (OECD), 2008. Guidelines for Testing of Chemicals: Guideline 425: Acute Oral Toxicity, OECD, Paris, France.
16. Adzu B, Haruna A, 2007. Studies on the use of Zizyphus spina-christi against pain in rats and mice. Afr. J. Biotechnol, 6(1), Pages-1317-24.
17. English M, 1996. Late-threatening severe malarial anemia trans. R. Soc Trop. Med. Hyg, 94, pp. 585-588.
18. Fidock DA, Rosenthal PJ, Croft SL, et al, 2004. Antimalarial drug discovery: efficacy models for compound screening. Nature reviews. Drug discovery, 3(6), pp. 509–20.
19. Bastidas O, 2013. Cell Counting with Neubauer Chamber (Basic Hemocytometer Usage), pp. 1–6.
20. Jimenez-Dıaz MB, Viera S, Ibanez J, et al, 2013. A New In-vivo Screening Paradigm to Accelerate Antimalarial Drug Discovery. PloS ONE, 8(6), pp. 12.
21. Ryley JF, Peters W, 1970. The antimalarial activity of some quinolone esters. Ann Trop Med Parasitol, 64(2), pp. 209-222.
22. Smilkstein M, Sriwilaijaroen N, Kelly JX, et al, 2004. Simple and inexpensive fluorescence-based technique for high-throughput antimalarial drug screening. Antimicrob Agents Chemother. 48(5), pp. 1803-1806.
23. Blanchard GB, Faustin KA, Félix TZ et al, 2018. Isocoumarins derivatives from Diospyros soubreana (Ebenaceae). EJBPS, 5(2), pp. 71-75.
24. Batista R, Ademir de Jesus Silva Júnior, Alaíde Braga de Oliveira, 2009. Plant-Derived Antimalarial Agents: New Leads and Efficient Phytomedicines. Part II. Non-Alkaloidal Natural Products, 14(8), pp. 3037–3072.
25. Saddiqa A, Usman M, Akmak OC, 2017. Isocoumarins and 3, 4-dihydroisocoumarins, amazing natural products, 41(2), pp. 153-178.
26. Khanh NX, de Vries PJ, Ha LD, et al, 1999. Declining concentrations of dihydroartemisinin in plasma during 5-day oral treatment with artesunate for falciparum malaria. Antimicrob Agents Chemother, 43(3), pp. 690–692.

How to cite this article

Nestor Tordzagla, Isaac Ayensu, Michael Fokuo Ofori, 2022. In vivo and in vitro antiplasmodial activity of extracts, fractions and an isolated compound from silk of zea mays (corn silk). J. Med. P'ceutical Allied Sci. V 11 - I 3, Pages - 4810 - 4816 doi: 10.55522/jmpas.V11I3.3160.