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In vitro antiplasmodial, cytotoxicity assay and partial chemical characterization of Kenyan *Physalis peruviana* L. (Solanaceae family) extracts

Peter Karanja Kamau[1*], Zipporah Ng'ang'a[1], Francis M. Njeruh[2] and John Thuita[3]

[1]Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya.
[2]Department of Public Health, Pharmacology, and Toxicology, University of Nairobi, P.O. Box 29053-00100, Nairobi, Kenya.
[3]Institute of Biotechnology research (Cancer division), Kenya Agricultural and Livestock Research Organization (KALRO), P.O. Box 362-00902, Kikuyu, Kenya.

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Malaria is a protozoan infection of Public health concern with several new cases yearly reported. Control of malaria infections is constrained due to the toxicity of currently available drugs and the emergence of resistant malaria strains. The current study was designed to assess the antiplasmodial activity, cytotoxicity and to partially characterize Kenyan *Physalis peruviana* extracts in order to determine their utility as a possible source of a new antimalarial drug. Antiplasmodial activity of *P. peruviana* extracts was evaluated *in vitro* using *Plasmodium falciparum* D6 chloroquine-sensitive, and W2 chloroquine-resistant by semi-automated microdilution technique. Cytotoxicity assay was determined using Vero cells; while partial characterization determined using Fourier transformer infra-red spectrophotometer (FTIR) and Gas chromatography-mass spectrophotometer (GC-MS). The antiplasmodial activity (IC_{50}) of *P. peruviana* extracts against chloroquine-sensitive (D6) *P. falciparum* strain ranged from 14.719±0.744 to >100 ug/ml. For W2, strain antiplasmodial activity ranged from 8.303±1.062 to >100 ug/ml. All the FTIR and GC-MS analysis of *P. peruviana* leave extract revealed the presence of biologically active components. There is a need for further studies using purified extracts as a means of coming up with possible novel antiplasmodial drugs. *P. peruviana* extracts were not toxic to Vero cells.

Key words: Antiplasmodial, cytotoxicity, Vero cells, *Physalis peruviana* extracts.

INTRODUCTION

Malaria is one of the most severe life-threatening protozoan diseases typically characterized by fever, paroxysm and flu-like symptoms recurring within 48 to 72 h cycles (Njokah et al., 2016). *Plasmodium falciparum* is by far the most virulent species of parasites that affect humans. In the 2017 World Health Organization (WHO) report on malaria, 216 million episodes of malaria and 445,000 deaths were reported, with 90% of cases and

*Corresponding author. E-mail: pkaranja@jkuat.ac.ke.

91% of deaths affecting the WHO African region (Kweyamba et al., 2019). Eradication of malaria has become a serious challenge due to the emergence of *P. falciparum* species resistant to the most potent recently developed antimalarial drugs, such as artemisinin-based combinations (WHO, 2007). As a result, there are limited options for the management of patients infected with such resistant parasites, a situation that necessitates renewed efforts to identify new chemical compounds with antimalarial activity.

Physalis peruviana L. belongs to the Solanaceae family. Botanically, *P. peruviana* can be classified as belonging to the Plantae Kingdom, the order solanales, the family Solanaceae, subfamily of solanoideae, tribe of physaleae, subtribe of physalinae and the species of *Physalis peruviana* (Sharma et al., 2015). The *P. peruviana* grown in Kenya is thought to have probable origin from tropical South America (Maundu et al., 1999) and is well adapted to Kenyan local environmental conditions. It is commonly found growing in the wild in Kenyan forests and in infertile soils where its fruits are collected and eaten. In Kenya, *P. peruviana* was reported to be used in herbal medicine against asthma (Njoroge and Bussmann, 2006), while in the Kagera region northwestern Tanzania, the fruit juice of *P. peruviana* was used as a cure for malaria (Moshi et al., 2012).

In the current study, the antiplasmodial activity and cytotoxicity of *P. peruviana* extracts were investigated. In addition, partial characterization of *P. peruviana* L. extracts was carried out with the aim of identifying the active compounds in the crude extracts with the highest anti-plasmodial activity.

MATERIALS AND METHODS

Study site, collection of *P. peruviana* and authentication

The *P. peruviana* plant materials were collected in February 2013 from Nyeri County [0°25`0``South, 36°57`0``East] located in the Central part of Kenya. Nyeri is 162 Km North of Nairobi. This area is known to have good reserves of *P. peruviana* L. The whole plant materials were collected in 2013 and identified by National Museums of Kenya Botanists and a Voucher specimen number EAH001PK deposited at the National Museums of Kenya Herbarium, Nairobi. Various parts; fruits, leaves, stem, and roots were separated, dried under shade and pulverized in a hammer mill fitted with a sieve of 0.5mm pores.

Antiplasmodial and cytotoxicity studies were carried out at the Centre for Biotechnology Research and Development (CBRD) Malaria Laboratory, Kenya Medical Research Institute (KEMRI) Headquarters, Nairobi, Kenya. Partial characterization of dichloromethane *P. peruviana* leaves extract was carried out at Jomo Kenyatta University of Agriculture and Technology chemistry laboratory, Nairobi, Kenya.

Preparation of *P. peruviana* aqueous, methanolic, and dichloromethane extracts

Preparation of *P. peruviana* extracts was carried out using methods described by Ubulom et al. (2011). Potions of pulverized plant parts were soaked separately in distilled water, methanol and dichloromethane, for 72 h with stirring at regular intervals. The extracts were repeatedly filtered using a sterile Whatman No. 1 filter paper (Jaca and Kambizi, 2011). The aqueous filtrates were freeze-dried, while the methanol and dichloromethane extracts were concentrated under vacuum at 40°C in a Buchii rotary evaporator.

The percentage yield was determined using the method used by Ogila (2010) as follows:

Percentage yield = (Weight of extract/Weight of ground material) X 100.

For identification purposes the extracts were assigned codes as follows; APPL = aqueous extracts of *P. peruviana* leaf; APPS = aqueous extracts of *P. peruviana* stem; APPF = aqueous extracts of *P. peruviana* fruit, APPR = aqueous extracts of *P. peruviana* root; MPPL = methanolic extracts of *Physalis peruviana* L leaves (MPPL), MPPS = methanolic extracts of *Physalis peruviana* L stem (MPPS), MPPF = methanolic extracts of *Physalis peruviana* L fruit (MPPF), MPPR = methanolic extracts of *Physalis peruviana* L root (MPPR), DPPL = dichloromethane extracts of *P. peruviana* leaf; DPPS = dichloromethane extracts of *P. peruviana* stem; DPPF = dichloromethane extracts of *P. peruviana* fruit; DPPR = dichloromethane extracts of *P. peruviana* root. All the extracts were kept desiccated at 4°C until use.

In vitro antiplasmodial bioassay

P. peruviana extracts were assayed using an automated microdilution technique to determine 50% growth inhibition of cultured parasites (Chulay et al., 1983; Desjardins et al., 1979).

Two different clones of *P. falciparum* were used in this study. The chloroquine-sensitive Sierra Leone 1 (D6) and chloroquine-resistant Indochina 1 (W2) strains were grown in a continuous culture supplemented with mixed gas (92% nitrogen, 5% carbon dioxide and 3% oxygen), 10% human serum and 6% hematocrit of O+ red blood cells and 5-fluorocytosine. Once cultures reached the optimum growth; growth rate ≥ 3.0% and parasitemia of > 3 % with at least an 80% ring developmental stage present, parasite solution was transferred to a 96 well microtiter plate with wells pre-coated with the test sample. The test samples were serially diluted across the plate to provide a range of concentrations used to accurately determine IC_{50} values. Plates were incubated in a gas chamber for 48 h after which ^{3}H-hypoxanthine was added and parasites allowed to grow for a maximum of 18 h. Cells were processed with a 96 well plate harvester (MicroBeta) onto filter mat paper (Wallac) and washed to eliminate unincorporated isotope. Filters were measured for *P. peruviana* antiplasmodial activity in a micro-titer plate scintillation counter (Wallac). Data from the counter was processed using the Oracle database program to determine IC_{50} values.

Cytotoxicity assays

The cellular cytotoxicity assay was used to test the cytotoxicity effects of individual extracts as was described by Wabwoba (2010) with slight modification. Briefly, Vero cells were seeded in minimum essential medium (MEM) (ATTC® 30-200™) supplemented with 10% Fetal bovine serum, 100 IU/ml penicillin and 100 µg/ml streptomycin in 25 ml cell culture flasks and incubated for 24 h at 37°C in 5% CO_2 humified atmosphere.

The Vero cells were harvested by trypsinization and pooled in 50 ml vials from which 100 µl suspensions at a concentration of 1 x 10^6 cells was added into 2 wells of rows A-H of a 96-well flat bottomed microtiter plate. A 100 µl of each *P. peruviana* extract was added and the plates incubated at 37°C in 5% CO_2 atmosphere. Cells without extracts and medium alone served as the controls. A 100 µl

Table 1. Percentage yields of aqueous, methanolic and dichloromethane *P. peruviana* extracts.

Plant extract	Amount of ground part in (g)	The yield of extract (g)	The yield of the extract (%)
APPL	100	10	10
APPS	100	3	3
APPF	120	0.6	0.5
APPR	100	6	6
MPPL	40	3.4	8.5
MPPS	100	5	5
MPPF	40	0.4	0.8
MPPR	100	5	5
DPPL	40	1	2.5
DPPS	40	1.34	3.4
DPPF	120	0.83	0.7
DPPR	120	4.3	3.6

of the cell suspension was discarded from each well followed with an addition of 10 µl of 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Roche, Indianapolis USA). After 4 h of incubation, the medium together with MTT was aspirated off from the wells, 100 µl of Dimethylsulphoxide added and plates shaken for 5 min. The absorbance was measured for each well at 562 nm using a microtiter plate reader (Wang et al., 2006).

Partial characterization of dichloromethane *P. peruviana* leaves extract by Fourier Transform infrared spectrophotometer (FTIR) and gas chromatography-mass spectrophotometry (GC-MS)

Fourier Transform Infrared Spectrophotometer (FTIR)

About 0.02 g of dried DPPL extract was reconstituted in dichloromethane and a drop of the extract mounted on NaCl plates for the FTIR analysis. The analysis was carried out using a Shimadzu 8400 FTIR with the scan ranging from 400 - 4000 cm^{-1}. The scanning was made through the IR region and the *P.peruviana* dichloromethane leaves extract functional groups determined according to the method described by Maobe and Nyarango (2013).

Gas chromatography-mass spectrometry analysis of dichloromethane leaves extract

Initially, 0.05 g of a homogenized DPPL extract was dissolved in 5 ml of dichloromethane in a centrifuge tube. The mixture was vortexed for 10 s and sonicated for 10 min. The mixture was centrifuged at 15000 *g* for 5 min. The supernatant was filtered before analysis in the GC-MS. Samples were analyzed using an Agilent 7890 Gas Chromatograph coupled to an Agilent 5975 Mass Spectrometer. The column used was the HP 5 MS capillary column: 30 m; 0.25 mm ID; 0.25 µm film thickness. The oven temperature program was initially set at 35°C and held for 3 min. It was then raised to 285°C at a rate of 10°C/ min. The oven was maintained at this temperature for 23 minutes. The total run time was 50 minutes. Mass spectrometry was done in full scan mode from 40 – 500 m/z with a solvent delay time of 3.5 minutes. Carrier gas was He (99.999%) at a flow rate of 1 ml/minute. One µL of the sample was injected using a split/splitless injector. The injector temperature was set at 200°C and the interface temperature was set at 280°C according to the method described by Wamalwa et al., (2015).

Statistical analysis

An independent t-test was performed on inhibition (IC_{50} in µg/ml) of *P. peruviana* extracts to both chloroquine-sensitive Sierra Leone 1 (D6) and chloroquine-resistant Indochina 1 (W2) strains. The level of significance was fixed at $P < 0.05$.

Ethical considerations

Permission to carry out the study was granted by the Kenya Medical Research Institute Ethical and Scientific Steering Committees (reference numbers KEMRI/RES/7/3/1 and ESACIPAC/SSC/101472 respectively).

RESULTS

Percentage yields of various *P. peruviana* extracts

The percentage yields for aqueous, methanolic, and dichloromethane *P. peruviana* extracts ranged from 0.5 to 10.0% (Table 1). *P. peruviana* aqueous leaves extract had the highest percentage yield, while the lowest percentage yield was recorded in the aqueous fruit extract.

Antiplasmodial assay of *P. peruviana* extracts

Most of the *P. peruviana* extracts exhibited various antiplasmodial activities. According to Rasoanaivo et al. (1992), antimalarial activity of plants is described as very active: IC_{50} <5 µg/ml, active: IC_{50} greater than 5 µg/ml but less than 50 µg/ml, weakly active: IC_{50} value greater than 50 µg/ml but less than 100 µg/ml and inactive: IC_{50} > 100 µg/ml. In this analysis, APPF and MPPF were the only ones found to be inactive (Table 2). An independent t-test

Table 2. *Physalis peruviana* aqueous, methanolic and dichloromethane extracts antiplasmodial activity (IC_{50}).

Extract	D6 IC_{50} (µg/ml)	W2 IC_{50} (µg/ml)
APPL	39.984±3.797	29.522±15.752
APPS	48.664±2.535	40.594±1.194
APPF	>100	>100
APPR	58.93±0.899	61.333±5.388
MPPL	36.479±1.519	20.855±6.044
MPPS	28.417±0.417	39.673±0.998
MPPF	>100 µg/ml	>100 µg/ml
MPPR	46.403±4.267	58.072±9.85
DPPL	14.719±0.744	8.303±1.062
DPPS	29.505±0.213	16.757±6.609
DPPF	66.752±1.098	32.945±18.282
DPPR	62.345±0.845	22.806±9.97
Chloroquine (ng/ml)	12.67±0.03	127.66±1.96

The IC_{50} values are represented as mean±SEM *P.peruviana* aqueous, methanol, dichloromethane extracts, and Chloroquine control drug against W2 and D6 *Plasmodium falciparum* strains.

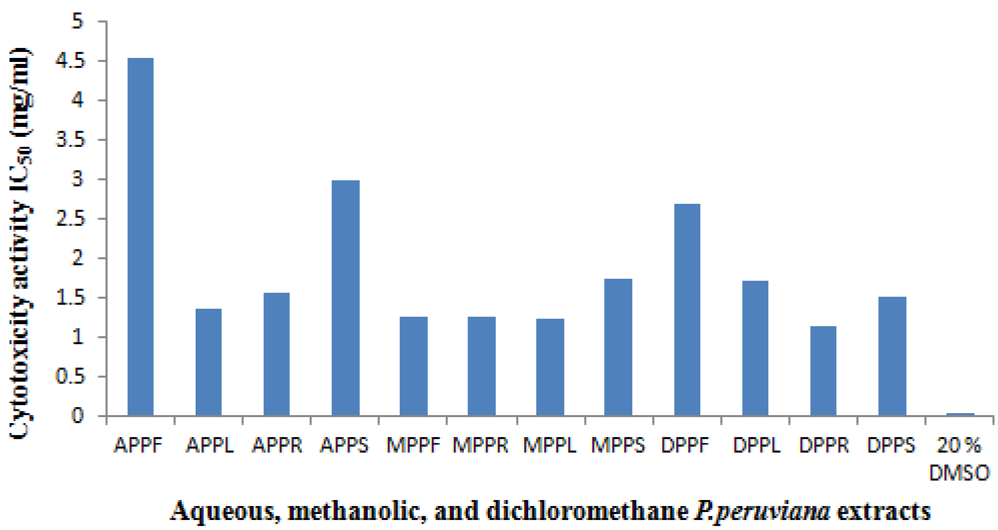

Figure 1. Cytotoxicity effects of aqueous, methanolic and dichloromethane *P. peruviana* extracts using Vero cells.

was performed to test the inhibition activity of the plant extracts against chloroquine-sensitive (D6) and chloroquine-resistant (W2) *P. falciparum* isolates. The inhibition activity of the extracts against D6 and W2 IC_{50} (µg/ml), t (22) = 0.016; P = 0.988, was not statistically different (P > 0.05).

Cytotoxicity effects of *P. peruviana* extracts

All the *P. peruviana* extracts were less toxic to Vero cells (higher IC_{50} values) as compared to the control, 20% DMSO (Figure 1).

Partial characterization of dichloromethane *P. peruviana* leaves extract FTIR and GC-MS

Fourier Transform Infrared Spectrophotometer (FTIR) analysis

The spectrum obtained indicated an intense absorbance

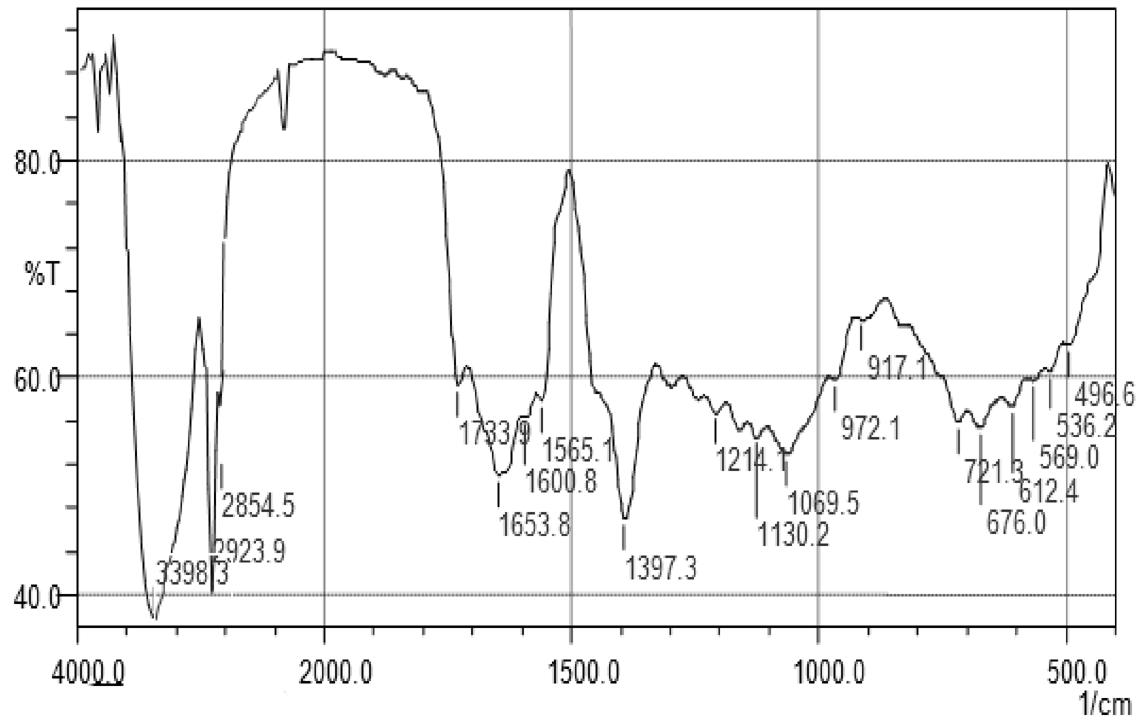

Figure 2. FTIR spectra of *P. peruviana* dichloromethane leaf extracts showing absorbance peaks.

at 3398 cm^{-1} attributed to O-H stretch vibrations in alcohol (Nithyadevi and Sivakumar, 2015). Polyphenols present in the plant extract is attributable to this absorbance value. The sharp shoulder peaks at 2923.9 and 2854.5 cm^{-1} are characteristic of C-H stretch in alkanes (Maobe and Nyarango, 2013). The small absorbance peak at 1733.9 cm^{-1} is attributed to the carbonyl C=O stretch in carboxylic acids, while the carbonyl stretch in ketones could be responsible for the absorbance peak at 1653.8 cm^{-1}. The absorbance peak at 1565.1 cm^{-1} due to N-H bending and peaks at 1130.2 and 1069.5 cm^{-1} attributed to C-N stretch vibrations in aliphatic amines could indicate the presence of alkaloids in the sample (Figure 2).

Gas chromatography-mass spectrometry analysis of *P. peruviana* dichloromethane leaves extract

Gas chromatography-mass spectrometry analysis of *P. peruviana* dichloromethane leave extracts revealed 8 phytochemicals with molecular weight ranges of 256 to 436 daltons. These phytochemicals included: Hexahydrofarnesyl acetone, n- Hexadecanoic acid, phytol, linoleic acid, ethyl iso-allocholate, vitamin E, campesterol and stigmasterol (Table 3).

The 8 Phytochemicals in Table 3 are also presented in the form of total ion current (TIC) (Figure 3).

DISCUSSION

The objective of the current study was to evaluate the antiplasmodial activity and safety of aqueous, methanolic and dichloromethane *P. peruviana* extracts, and also to partially characterize the extract exhibiting the highest antiplasmodial activity.

The yield of aqueous, methanol and dichloromethane *P. peruviana* L. extracts ranged from 0.5 - 10, 0.8 - 8.5 and 0.7 - 3.6% respectively. These results were comparable to those of other researchers who reported that the more polar solvents yielded greater quantities of extracts (Gaba et al., 2019; Abubakar, 2010).

From this study 22 (91.7%) out of the 24 *P. peruviana* extracts exhibited antiplasmodial activity against the two laboratory *P. falciparum* clones, chloroquine-sensitive D6 (Sierra Leone) and chloroquine-resistant W2 (Indochina). The mean IC_{50} (µg/ml) for the antiplasmodial activity for *P. peruviana* extracts against the D6 clone ranged from 14.719±0.744 to > 100 µg/ml, while the mean IC_{50} of *P. peruviana* extracts ranged from 8.303±1.062 to > 100 µg/ml. The DPPS extract exhibited the highest activity for both clones while the weakest antiplasmodial activity was from APPF and MPPF both at > 100 µg/ml. According to Rasaonaivo (1992), the classification of antiplasmodial activity on crude plant extracts, APPF and MPPF could, therefore, be classified as inactive. These results are in agreement with a study carried out by Moshi (2012) in the Kagera region, northwestern Tanzania which found that

Table 3. Phytochemicals of *P. peruviana* dichloromethane leaves extract by GC-MS analysis.

Peak number in the sample TIC	Retention time (min)	Compound name	Molecular weight (Daltons)	Reported Activity	References/ literature
2	22.635	Hexahydrofarnesyl acetone	268	Biologically active	Nikkhah et al. (2017)
3	24.180	n-Hexadecanoic acid	256	Antioxidant, anticancer	Nikkhah et al. (2017)
4	25.323	Phytol	296	Antimicrobial, anti-inflammatory, Anticancer, diuretic	Nibret and Wink (2010)
5	25.905	Linolenic acid	278	Cytotoxic, antitrypanosomal	Nikkhah et al. (2017)
6	27.518	Ethyl iso-allocholate	436	Antifungal	Mohan et al. (2012)
7	35.289	Vitamin E	430	Antioxidant	Malathi et al. (2016)
8	37.126	Campesterol	400	Anti-CDV	Choe and Min (2009)
9	37.776	Stigmasterol	412	Anti-osteoathritic	Genser et al. (2012)

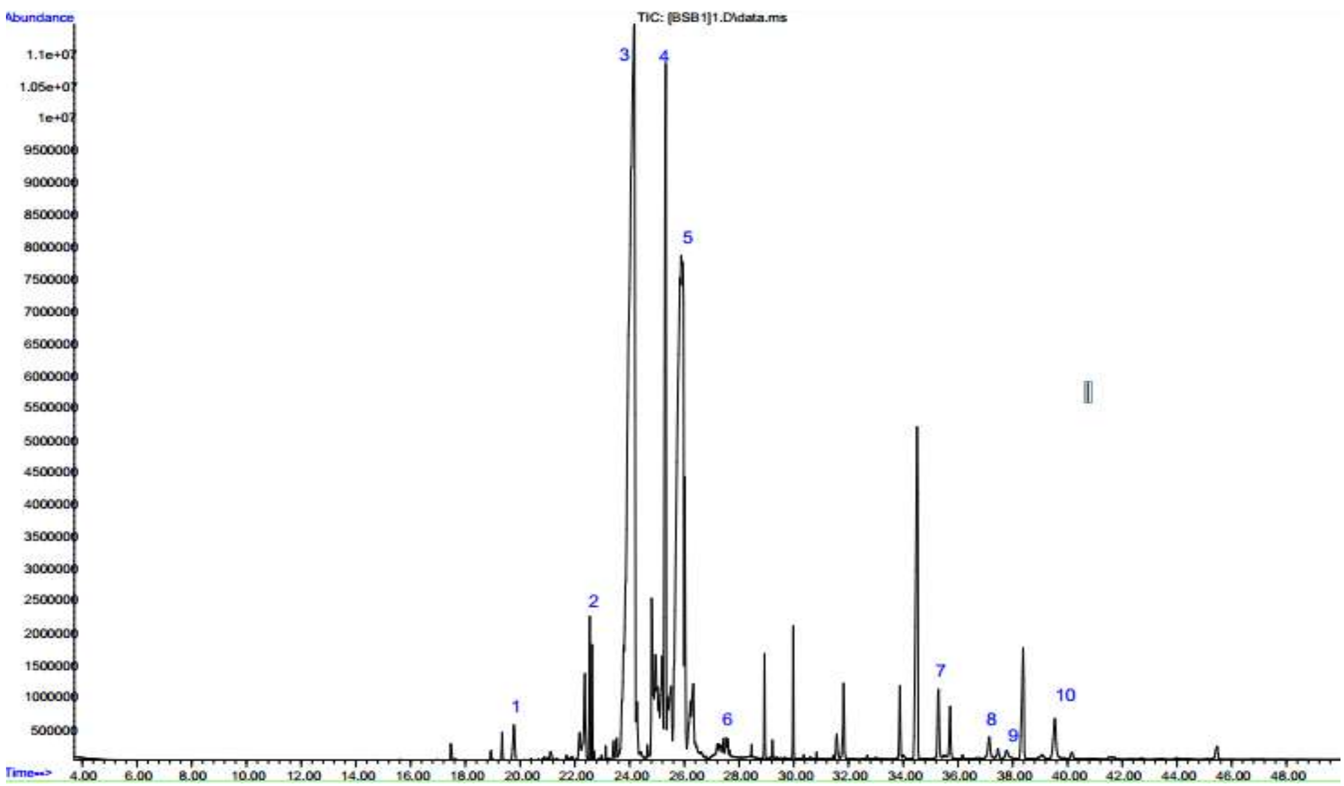

Figure 3. Total Ion Current (TIC) for *P. peruviana* leaves dichloromethane extract.

P. peruviana was being used to treat malaria as herbal medicine. The results are comparable to an *in vitro* study carried out by N'guessan et al. (2010) on *P. angulata* a similar species to *P. Physalis* that exhibited antiplasmodial falciparum activity of IC_{50} (µg/ml) of 7.9±0.3. These results are quite close to antiplasmodial activity exhibited by dichloromethane *Physalis peruviana* L extracts in the current study.

It was necessary to determine the safety profile of plant extracts due to the complexity and natural biological variations emanating from them (Cowan, 1999). Cytotoxicity effects of *P. peruviana* extracts using Vero cells revealed that all extracts were less toxic compared to the control. However, according to the US National Cancer Institute (NCI), *P. peruviana* extracts tested were not within the toxic group of IC_{50} < 20 µg/ml in the preliminary assay (Nathyadevi and Sivakumar, 2015).

The FTIR is an invaluable tool for the characterization and identification of phytochemicals or functional groups present in an unknown mixture of plant extract (Maobe and Nyarango, 2013). The spectrum obtained indicated the presence of O-H stretching for alcohols, Polyphenols, C-H stretch for alkanes, the carbonyl C=O stretch for carboxylic acids, and the carbonyl stretching for ketones, N-H bending and peaks at 1130.2 cm^{-1} and 1069.5 cm^{-1} attributed to C-N stretch vibrations in aliphatic amines could indicate the presence of alkaloids in the sample. The presence of these functional groups could be attributed to the medicinal properties as was revealed from a previous study (Maobe and Nyarango, 2013).

Gas chromatography-mass spectrometry revealed the presence of some compounds with biological activities such as Hexahydrofarnesyl acetone, n-Hexadecanoic acid, Phytol, Linolenic acid, Ethyl iso-allocholate, vitamin E, campesterol and stigmasterol. Linoleic acid, Phytol and Ethyl isoallocholate have been associated with antimicrobial activities (Nibret and Wink, 2010; Mohan et al., 2012; Malathi et al., 2016), which could have been attributed to the antiplasmodial activity of *P.peruviana* extracts. In a study carried out by Kamau et al. (2017), *P. peruviana* extracts were found to contain biologically active compounds such as tannins, saponins, steroids flavonoids, and alkaloids. Alkaloids have been associated with the antiplasmodial activity (Wright and Philipson, 1990).

Conclusion

Aqueous, methanolic and dichloromethane *P. peruviana* leaves, stem, fruits, and roots extract exhibit antiplasmodial activity against *P. falciparum*. The ability of 92% of *P. peruviana* extracts to kill chloroquine-resistant Indochina 1 (W2) strains is of importance where chloroquine-resistant malaria is in existence. The investigations demonstrated the effectiveness and safety of using herbal treatment of malarial by *P. peruviana* by the rural indigenous communities. Partial characterization of *P. peruviana* extracts by FTIR and GC-MS revealed biologically active phytochemicals that could be attributed to the antiplasmodial activity exhibited by *P. peruviana* extracts. The study recommends the use of purified extracts and elucidation of actual compounds against *P. falciparum* activity.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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