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Antiplasmodial activity, phytochemical analysis and in silico study of the leaf extract of *Chenopodium opulifolium* Koch & Ziz used in Uganda

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Abstract

The plant *Chenopodium opulifolium* is traditionally consumed as a food and for treating ailments. Despite its use in Uganda for treating malaria, there are no scientific studies on its phytochemicals and biological potential to ascertain its folkloric claims. In this study, bioassay (antiplasmodial activity) – guided fractionation, isolation and identification of the phytoconstituents of the leaves of *C. opulifolium* were carried out. The identified compounds were further investigated via in silico docking to propose the mechanism of action of the extracts.

The leaves were extracted sequentially via maceration with n-hexane (HEX), dichloromethane (DCM), ethyl acetate (EtOAc), methanol (MeOH), and water. The most active MeOH and DCM extracts were fractionated via chromatography and the compounds were identified via 1D and 2D NMR, FTIR, and GC-MS techniques. The extracts and isolated compounds were tested against the *P. falciparum* 3D7 strain and clinical field isolates KOM_234 and KDH_204 via a fluorescence-based SYBR Green 1 assay technique. Docking was performed in the active and nucleoside binding sites of the plasmodial lactate dehydrogenase PfLDH.

The five extracts showed activity against the *P. falciparum* 3D7 strain. The MeOH and DCM extracts were the most active with IC₅₀ values of 0.1177 and 1.189 µg/mL respectively. In *P. falciparum* clinical field isolates KOM_234 and KDH_204, the MeOH extract had IC₅₀ values of 5.30 µg/mL and 5.06 µg/mL respectively whereas allantoin from the MeOH extract had an IC₅₀ value of 2.29 µg/mL and 10.94 µg/mL respectively. Among the identified and isolated compounds 2-myristinoyl pantetheine showed the best binding affinity (-8.3674) for plasmodial dehydrogenase (chloroquine = -6.3451). The antiplasmodial activities of the extract and the identified compounds of *C. opulifolium* leaves rationalize the traditional use of the plant in malaria therapy. The activity of the extracts can be attributed to the collective constituents principally 2-myristinoyl pantetheine which binds to PfLDH.

Keywords *Chenopodium opulifolium*, antiplasmodial activity, malaria, phytochemicals, in silico analysis

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Introduction

The disease burden of Malaria is enormous and it is one of the world's deadliest infectious diseases caused by *Plasmodium* species including *P. falciparum* which distresses the largest population [1]. In 2021, 227,000,000 infections and 619,000 cases of malaria mortality were confirmed worldwide. Morbidity and mortality rates are 95% and 96% respectively, in sub-Saharan Africa [1]. Additionally, malaria in Uganda is the greatest cause of illness and death [2].

There have been several advances in many areas to control malaria [3]. These include developing effective vaccines, regulating mosquito vectors, and identifying new chemotherapeutic agents, but morbidity and mortality remain unsatisfactorily high [3]. Many effective antimalarial drugs such as chloroquine, pyrimethamine/ sulfadoxine, artemisinin, amodiaquine, quinine, mefloquine, artemether, lumefantrine have been discovered, and they indisputably limited both morbidity and mortality [4]. However, there is increased resistance to conventional antimalarial drugs, making malaria treatment difficult. In addition, the unattainability and unaffordability of these drugs; amplified resistance of mosquito vectors to insecticides; and adverse effects of existing antimalarial drugs, all of which pose considerable challenge in treating and minimizing death due to malaria [5].

For centuries, people have been treating malaria with medications from nature [6]. These include quinine from cinchona bark and recently artemether synthesized from artemisinin which was isolated from *Artemisia annua* [7]. In Uganda, approximately 90 % of the population, especially women and children, are dependent on herbal medicines [8, 9].

Amaranthaceae is one of the major group angiosperm family and comprises largely halophytic herbs, infrequently shrubs or trees comprising about 102 genera and more than 2000 species [10]. *Chenopodium* is the principal genus of this family and comprises about 150 species including *Chenopodium opulifolium* with almost worldwide distribution [11]. The utmost representative constituents are primary metabolites like carbohydrates, amino acids, nonpolar constituents, proteins, aromatic cytokines, hormones and secondary metabolites like flavonoids, saponins, terpenes, sterols, alkaloids, vitamins, phenolic compounds, ecdysteroids and triterpenoids [12] and are reported to possess numerous medicinal properties used in folk medicine. Current pharmaceutical research has proven their potent antipyretic, antibacterial, antiviral, antifungal, antimalarial, anthelmintic, antioxidant, trypanocidal, antineoplastic, immunomodulatory and anticancer properties [12].

Chenopodium opulifolium is a grey erect annual or short-lived perennial herb, with spreading branches, often woody, height below 60–150 cm tall. The plant is

occasionally harvested for local use as food and medicine [13] and is native to Central and Southern Europe, Northern and Eastern Africa, Arabian Peninsula to Western and Central Asia [10]. *C. opulifolium* is a very important plant in Uganda for malaria treatment, and is commonly known locally as Omwetango in central and western Uganda [14, 15]. The plant is used by the local community mainly to treat malaria [14, 15] and other illnesses like cough, measles, vomiting, HIV/AIDS, eye infections, sore throat, managing pediatrics, cases of febrile illnesses, induce menstruation, diarrhea, hasten childbirth and to relieve irregular painful menstruation [8, 16, 17, 9, 18–20]. In Democratic Republic of Congo, the herbal extract is prepared by decoction, infusion or maceration and administered orally and topically to treat malaria, dysentery, gastrointestinal disorders, hyperglobulinemia, intestinal worms, leukemia, measles and shrinkage of the vagina [21]. It was also mentioned 27 times among the local communities of Bwambara sub-county in Rukungiri District, Western Uganda for treatment of herpes zoster, liver disease, allergy, tinea capitis, worms, strange traditional diseases, oath, burns/diseases caused by traditions of twins and sexual dysfunction [22]. Ear infections are treated by the plant by infusion and persistent fevers by bath by the people in the Districts of Arua, Dokolo, Mbale, Iganga, Bushenyi, Rakai, Luwero and Kaabong in Uganda [23]. The leaves extract possesses significant antioxidant, antinociceptive and anti-inflammatory effects due to presence of bioactive compounds like alkaloids, tannins, phlobatannins, flavonoids, and saponins [24]. The leaves extract also exhibits immunomodulatory [25] and hypoglycemic activity [26]. Acute toxicity test on the aqueous extract of *C. opulifolium* leaves showed that it is nontoxic and safe to be used [24, 26].

However, the efficacy of *C. opulifolium* and its chemical constituents against *Plasmodium* species has not been scientifically validated. Considering several claims by traditional therapists that malaria can be treated with herbal medicines such as *C. opulifolium*, this can provide an alternative source for antimalarial drugs [14, 15]. Owing to the extensive use of this plant for treatment, the current study investigated the antiparasmodial activity of the leaves as well as isolation, characterisation, in silico study and the identification of phytochemical constituents of *C. opulifolium*.

In addition to validation of its antimalarial activity, the use of in silico techniques such as molecular docking provided insights into the mechanism of action of the identified constituents. *Plasmodium falciparum* lactate dehydrogenase (PfLDH) which is involved in glycolysis has been regularly used as a representative target for in silico antimalarial screening. This target is known to have 2 important binding sites namely the enzyme active site, and the cofactor binding site [27]. Structures

from the PDB database (1U5A and 1CET) showing different cocrystallized ligands for the respective sites were employed. The identified compounds with molecular weights less than 500 Da were docked into both sites to investigate the interactions therein.

Materials and methods

General experimentation

The melting points were determined by melting point equipment (Bibby Stuart Scientific). The solvents were evaporated via a Buchi Rotary vacuum evaporator (BUCHI-R 205) [organic solvents] and freeze drier (Alpha 1-2LDplus) [aqueous solvents]. Chromatographic analysis (TLC) was achieved via precoated slides of aluminum foil with silica gel (0.2 mm thickness, Kiesegel 60 F₂₅₄, Merck type), which were observed via UV, iodine vapor and anisaldehyde sprays. Gravity column chromatographic investigations were performed using silica gel (Fluka) 60–120 mesh [28]. Phytochemicals were investigated via reported procedures in the literature [29, 30, 31]. Infrared spectroscopy (IR) spectra were recorded on a Perkin-Elmer Spectrum using a Fourier transform spectrophotometer with the samples run as discs in KBr and the absorbance was recorded at a scan range of 4000–400 cm⁻¹. The GC-MS investigation was carried out via Agilent Technologies GC-MS where a database from library version (2005), NIST, software, Turbomas 5.2, was used for interpretation. ¹H, ¹³C, and two-dimensional NMR (COSY, HSQC, and HMBC) were run at 500/600 MHz in deuterated chloroform / DMSO-d₆ to obtain the spectral data, which were used to simulate the structures and MNOVA14.2.1 software was used [28].

Materials

Chemicals and reagents

n-hexane, DCM, EtOAc, MeOH, chloroform and petroleum ether all laboratory grade reagents (Sigma-Aldrich), Whatman filter paper (Whatman® No. 1), aluminum foil with silica gel (0.2 mm thickness, Kiesegel 60 F₂₅₄, Merck type), and silica gel (Fluka) 60–120 mesh.

Equipment

Melting point equipment (Bibby Stuart Scientific), Rotary vacuum evaporator (BUCHI-R 205), freeze drier (Alpha 1-2LDplus), Perkin-Elmer Spectrum using a Fourier transform spectrophotometer, Agilent Technologies GC-MS (GC-7890 A, MS 5975 CB.07.02.1938 08- Sep-2014 Copyright © 1989–2014 Agilent Technologies, Inc.) NIST library version (2005) software, ¹H, ¹³C, and two-dimensional NMR (COSY, HSQC, and HMBC) (Bruker, 500/600 MHz), MNOVA14.2.1 software and Molecular Operating Environment software (MOE 2015).

Plant leaf material

C. opulifolium plants (Omwetangu in Luganda) were collected on basis of ethnobotanical descriptions with the help of local traditional healers on April 2019 from Mbirizi, Masaka District, Uganda. Taxonomists at Makerere University (Mr. Protase Rwaburindore) identified and authenticated the plant samples, gave accession number 51,011 and voucher number 12, and then placed them at the Makerere University Herbarium. The leaves were detached, cleaned with distilled water, dried under shade for 21 days [29], milled into a fine powder via an electric motor, and stored in a well-enclosed vessel.

Extraction

The dried plant sample (600 g) was macerated sequentially using 3.0 L each of *n*-hexane, DCM, EtOAc, and MeOH from laboratory-grade reagents with intermittent agitation every 6 h at room temperature for 24 h repeatedly and exhaustively with total volumes of 9.9, 8.4, 12.7 and 14.22 L respectively. Distilled water (2.5 L) was used for the aqueous extraction of the sample (150 g). The filtered extracts were concentrated to dryness and then stored below 4 °C until further analysis. The yield percentages were determined on a dry weight/weight basis.

Bioassay-guided fractionation

The second most active methanol extract (23.22 g) was fractionated on a silica column using chloroform/methanol with a polarity increase from 0 to 100% methanol, then methanol/water combination with a polarity increase from 0 to 50% water, and then a water/acetic acid combination in the final wash to yield 20 fractions; M1-M20. M12 to M16 were combined on the basis of TLC analysis, washed with methanol and then recrystallized with methanol/water to give a white crystalline solid (Compound 1). TLC of compound 1 in chloroform/methanol/water (2:8:0.5) revealed a single spot visible as a faint white color under UV (λ = 254 nm) and not visible in iodine vapor, R_f = 0.74. The NMR, IR, and melting point were determined to yield compound 1 (550 mg).

The most active dichloromethane crude extract (10 g) was fractionated starting with 100% hexane and 0% ethyl acetate while increasing the polarity to 100% ethyl acetate and then washed with methanol resulting into 27 fractions; F1-F27 where fractions F4 and F5 were pooled and combined (0.91 g); and fractionated with 80% petroleum ether: 20% ethyl acetate combined and then 20–100% ethyl acetate increased, after which then 0–100% methanol increased, leading to nine fractions; D1-D9. The white solids obtained from D1, D4, D5, D6, and D7 were combined on the basis of their TLC results and washed with petroleum ether to obtain white powders. TLC of this powder in chloroform/methanol/water (9:1:0.5) revealed a single spot, R_f = 0.91 and on the basis

of spectral analysis, it was identified as compound 2 (133 mg). GCMS analysis was carried out on fractions D1 and D7, and 16 compounds were identified from the fractions.

Bioactivity

Antiplasmodial activity was carried out at Noguchi Memorial Institute for Medical Research against the *Plasmodium falciparum* chloroquine (CQ)-sensitive 3D7 strain and against the clinical field isolates KOM_234 and KDH_204 from Kenya Medical Research Institute.

All extracts and compounds were tested via nonradioactive Malaria SYBR Green assay technique [32], where the IC_{50} value of various parasite cultures were determined. These parasites were grown as previously described by Trager & Jensen, 1976 [33]. The preparation of the medium (RPMI 1640) was performed as described by Cheruiyot et al., 2016 [34].

Stock concentrations of 20 mg/mL of compounds/extracts were prepared (in 0.5% final DMSO), and 10-fold serial dilutions (in culture media) of the compounds were prepared from 20,000 to 0.02 μ g/mL to obtain seven dissimilar concentrations (20,000, 2000, 200, 20, 2, 0.2 and 0.02 μ g/mL).

The compounds (50 μ L) were added in sextuplicate to a 96-well culture plate where chloroquine, mefloquine, and artesunate (10-fold dilutions from 2000 ng/mL 0.002 ng/mL) were used as positive controls, and CPM was used as a no drug control. To the first triplicates, 50 μ L of a 1% parasitemia and 4% hematocrit synchronized ring stage culture mixture was added, while 50 μ L of 4% hematocrit uninfected RBCs was added to the second set of triplicate wells. The plates were gassed for 6 min after which they were incubated at 37 °C for 72 h. Then the plates were harvested, wrapped in aluminum foil, and frozen at -20 °C for 24 h. The plates were thawed at room temperature, and 100 μ L of lysis buffered SYBR Green I (1 μ L of SYBR Green I in 10 ml of lysis buffer) was added to each well and incubated at room temperature for 1 h. The fluorescence was measured at excitation wavelength of 497 nm and an emission wavelength of 530 nm. The IC_{50} values were determined from the dose-response curve, where the results are expressed as the mean $IC_{50} \pm$ standard deviation (SD) determined from four independent experiments. The two most active extracts were subjected to chromatographic separation to isolate the active compounds.

Molecular docking simulation

Target preparation

This was performed in Molecular Operating Environment software (MOE 2015) [35]. The pdb files of PfLDH (PDB IDs: 1U5A and 1CET) were downloaded from the RCSB Protein Data Bank (<https://www.rcsb.org/>) and

imported into MOE. The automatic QuickPrep function was used to prepare the proteins. This involved the correction of structural issues, 3D protonation, and the removal of water molecules farther than 4.5 Å from the ligand or receptor. Finally, minimizations were performed until an RMS gradient of 0.01 kcal/mol/Å was obtained. The prepared proteins were saved as MOE molecule files (.mol).

Ligand preparation

The canonical SMILES notations of the compounds were copied from PubChem into MOE to generate 3D structures. The molecules were then energy minimized by applying forcefield MMFF94 and saved as an MOE database file (.mdb).

Docking protocol

Docking studies were performed via molecular operating environment (MOE) software. The MMFF94 forcefield and partial charges were automatically calculated. The alpha triangle placement was chosen to determine the poses. The free energy of ligand binding from a given pose was estimated by the London ΔG scoring function. The London ΔG scoring function was used for rescoring after the results were refined. The output was saved as a database dock file with different poses for each ligand on the basis of the final score function (S). The cocrystallized ligands, -3,5-dihydroxy-2-naphthoic acid and chloroquine for 1U5A and 1CET respectively, were redocked back to their putative binding sites to validate the docking protocol [35].

Validation of docking protocol

The co-crystallized ligands, -3,5-dihydroxy-2-naphthoic acid and chloroquine for 1U5A and 1CET respectively, were re-docked back to their putative binding sites to validate the docking protocol [35]. The redocking was performed using the docking protocol above and the RMSD between the co-crystallized model and that generated by the docking protocol was calculated.

Results and discussion

Plant material extraction

The hexane, DCM, EtOAc, MeOH, and water extracts produced whitish, black, black, reddish brown, and dark brown solids with percentage yields of 1.59%, 2.31%, 0.83%, 4.53%, and 28.37% respectively. The high aqueous extract yields meet the medicinal needs for which they were prepared. The composition of the plant extract revealed that the percentage of polar compounds was greater than that of non-polar compounds.

Phytochemical screening of organic and aqueous leaf extracts of *Chenopodium opulifolium*

Analysis of phytochemicals revealed phytochemicals present in *C. opulifolium* (Table S1 [Supplementary data]). Both aqueous and organic leaf extracts of *C. opulifolium* were found to contain alkaloids, steroids, coumarins, phlobatannins, reducing sugars, flavonoids, tannins, saponins, phenolic compounds and terpenoids. Glycosides, cardiac glycosides, and anthraquinones were not present in the tested extracts. The methanol extract had the greatest number of phytochemicals, followed by the aqueous extract, ethyl acetate extract, DCM extract and hexane extract.

Phytochemical analysis revealed that most compounds from *C. opulifolium* leaf extracts are polar. Compared with previous studies, some of the phytochemicals were reported in *C. opulifolium* aqueous leaf extracts, which is in total agreement with the findings of this research [24].

Table 1 Phytochemical components detected in the DCM extract of *Chenopodium opulifolium* leaves, including their chemical formula

S. No.	Compound name	RT	MF	MW
1.	Dihydroxanthin	4.189	C ₁₇ H ₂₄ O ₅	308.4
2.	m-Toluidine, N-trimethylsilyl-N-(Trimethylsilyl)-m-tolylamine	7.18	C ₁₀ H ₁₇ Nsi	179.33
3.	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-en-1-yl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	19.689	C ₂₃ H ₃₂ O	324.5
4.	Alpha-hydroxy-alpha-(p-(dimethylamino) phenyl) acetophenone	21.12	C ₁₆ H ₁₇ NO ₂	255
5.	Tridecanoic acid	21.38	C ₁₃ H ₂₆ O ₂	214
6.	Ethyl iso-allocholate	29.311	C ₂₆ H ₄₄ O ₅	436
7.	Amphibine D	34.871	C ₃₆ H ₄₉ N ₅ O ₅	631
8.	Papaveroline, 2'-bromo-2-methyl-, tetramethyl (ester)	46.855	C ₂₁ H ₂₆ BrNO ₄	436
9.	(1aR)-3-[(Acetyloxy)methyl]-1aα,1bβ,4,4a,5,7aα,7b,8,9,9a-decahydro-1,1,6,8α-tetramethyl-1 H-cyclopropa[3,4]benz[1,2-e]azulene-4aβ,5β,7ba,9β,9aα-pentol 5,9,9a-triacetate	48.108	C ₂₈ H ₃₈ O ₁₀	534
10.	2-Myristinoyl pantetheine	9.754	C ₂₅ H ₄₄ N ₂ O ₅ S	484.7
11.	Isoquinoline, 1-[(3,5-dihydroxy)benzyl]-1,2,3,4-tetrahydro-6-hydroxy - HIGENAMINE	18.372	C ₁₆ H ₁₇ NO ₃	271.31
12.	Oxiraneundecanoic acid, 3-pentyl, methyl ester, trans	20.135	C ₁₉ H ₃₆ O ₃	312
13.	7(1 H) Pteridinone	21.12	C ₆ H ₄ N ₄ O	148
14.	Dodecanoic acid	21.298	C ₁₂ H ₂₄ O ₂	200
15.	Oleic Acid	22.081	C ₁₈ H ₃₄ O ₂	282
16.	Hexanedioic acid mono (2-ethylhexyl) ester	29.311	C ₁₄ H ₂₆ O ₄	258

RT Retention time, MF Molecular formula, MW Molecular weight

Identification of compounds from fractions of dichloromethane extract via GC-MS analysis

Sixteen (16) major compounds were identified from dichloromethane fractions D1 & D7 and are represented in Table 1. They encompass many biological activities. Ethyl isoallocholate possesses anti-inflammatory, antioxidant, antihepatotoxicity, neuroprotective, anticancer and antimicrobial activities [36]. Oleic acid has antifungal, antioxidant, and mosquito larvicidal activities [37–39]. Dodecanoic acid has antioxidant, free radical scavenging, and hepatoprotective activity [40, 41]. Tridecanoic acid has antioxidant and antimicrobial activities [42]. The bio-activity/ antiplasmodial activities of the rest remaining compounds are not known.

Structural elucidation of compounds 1 and 2

Allantoin [(2, 5-dioxo-4-imidazolidinyl) urea] (1)

White crystalline; melting point: 233 °C (literature result: 234–235 °C reported) [43, 44]; IR (KBr) 3436.43, 3340.25, 2761.95, 1325.16, 1183.29 and 823.42 cm⁻¹; ¹H NMR (in DMSO-d₆, 600 MHz, δ, ppm, J/Hz); 10.52 (1H, s-H-5), 8.03 (1H, s-H-4), 6.87 (1H, d, J = 8.2 Hz, H-3), 5.76 (2 H, s, H-2), 5.25 (1H, d, J = 8.1 Hz, H-1); ¹³C NMR (600 MHz, DMSO-d₆, δ, ppm); 173.53 (C4), 157.32 (C3), 156.71 (C2), 62.40 (C1), 39.52 (DMSO-d₆ signal).

Compound 1 (Fig. 1) was obtained as white crystals, and decomposed at 233 °C. The FTIR (KBr) spectrum revealed a medium to strong vibration band peaks at 3436.43, 3340.25, 1360.37, 1325.16, 1183.29 and 823.42 cm⁻¹ which indicated the presence of NH (stretch), RCONH₂ (stretch), NH₂ (stretch), NH₂ (scissoring), amide/cyanide stretching and NH₂ (wagging on hydrogen bonding) groups respectively. The ¹H NMR (600 MHz, DMSO-d₆, δ, ppm, J/Hz) spectrum revealed 5 signals which clearly defined six protons with one broad amino (NH₂) group signal (singlet) present at 5.76 ppm attached to a carbonyl. The HSQC spectrum confirmed the direct attachment of the methine proton at 5.25 ppm to the saturated carbon at 62.40 ppm (Table 2). The three distinct carbon signals for the carbonyl groups present at δ_C: 156.71, 157.32, and 173.53, and one carbon signal at δ_C: 62.40 account for the methine group and were revealed by the ¹³C-NMR data.

All the assignments were confirmed via COSY and HMBC experiments and when cross-checked with the literature, compound 1 was confirmed to be allantoin [43, 44 & 45].

It is an imidazole alkaloid which is in total agreement with phytochemical analysis confirming that alkaloids are positive in the methanol extract. Although this compound was reported in *C. opulifolium* for the first time, several studies have confirmed the isolation of Allantoin from African *Cricetidae* urine [46], and from plants such as the lichens *Umbilicaria esculenta* [47], *Trichosanthes*

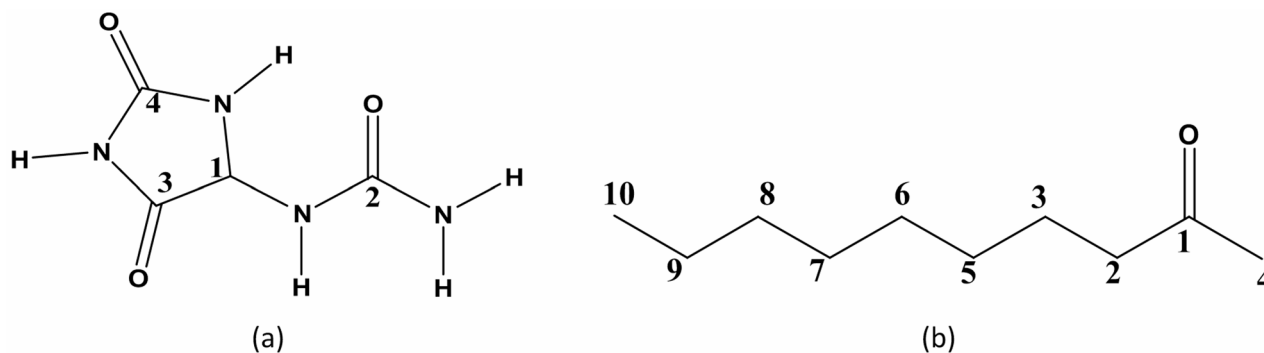


Fig. 1 The structure of the two isolated compounds: **a** compound 1; **b** compound 2

Table 2 ^1H and ^{13}C NMR (in DMSO- d_6) spectroscopic data of compound 1 compared with literature values [43]

^1H atom	$^1\text{H/ppm(J)}$ (600 MHz)	$^1\text{H NMR}$ (400 MHz) literature	^{13}C Atom	$\delta^{13}\text{C/ppm}$ NMR (600 MHz)	$\delta^{13}\text{C/ppm}$ NMR (100 MHz) literature	COSY	HMBC
H1	5.25 (1 H, doublet, $J=8.1$) Hz)	5.23	C1	62.40	62.3	H3	C3, C4
H2	5.76 (2 H, singlet)	5.78	C2	156.71	156.6		C1
H3	6.87 (1 H, doublet, $J=8.2$) Hz)	6.87	C3	157.32	157.2	H1	C1, C3, C4
H4	8.03 (1 H, singlet)	8.05	C4	173.53	173.5		C1,C2,C3,C4
H5	10.52 (1 H, singlet)	10.53					C1, C3, C4

Table 3 ^1H and ^{13}C NMR spectroscopic data (500 MHz, CDCl_3) for compound 2 compared with literature values [59] [^1H NMR]

Atom	$\delta^{13}\text{C/ppm}$	$^{13}\text{C}_{\text{atom}}$	$^1\text{H/ppm(J)}$	$^1\text{H/ppm(J)}$ -Literature
1	207.3	CO		
2	43.9	CH_2	2.34(2 H, t, $J=4.1$)	2.37 (2 H, t, $J=7$)
3	32.9	CH_2	1.50(2 H, m)	0.7–1.8
4	31.9	CH_3	2.10(3 H, s)	2.02 (3 H, s)
5	30.9	CH_2	1.20(H, m)	0.7–1.8
6	29.6	CH_2	1.20(2 H, m)	0.7–1.8
7	29.3	CH_2	1.20(2 H, m)	0.7–1.8
8	25.7	CH_2	1.20(2 H, m)	0.7–1.8
9	22.7	CH_2	1.20(2 H, m)	0.7–1.8
10	14.3	CH_3	0.89(3 H, t, $J=3.8$)	0.7–1.8

and carbonyl groups were both represented by infrared (KBr) spectra at 2916.52, 2848.58, 1462.85 cm^{-1} and 1736.75 cm^{-1} respectively and ^{13}C NMR data at δC : 14.3 & 31.9 [Methyl], methylene (δC : 43.9, 32.9, 30.9, 29.6, 29.3, 25.7 & 22.7) and carbonyl (δC : 207.3 ppm) are represented in Table 3. In the analysis of the spectral data and comparison with the reported literature, compound 2 was identified as decan-2-one (Fig. 1). It was previously identified in essential oil from *Houttuynia cordata* which contains 4.99% of 2-decanone [56]; and the essential oil of *Zanthoxylum alatum* which contains 20.85% 2-decanone as one of the main components [57]; and the essential oil of fringed *Ruta chalepensis* [58].

kirilowii [48], seeds of *Lithospermum erythrorhizon* [49], *Protea compacta* seeds [50], yam tubers [51], BuOH fraction of bitter melon (the fruits of *Momordica charantia*) [52] leaves of *Palicourea coriacea* (Cham.) K. Schum [53], stems of *Aristolochia gigantea* [54], *Portulaca oleracea* seeds [55], and many other plants.

Decane-2-one (2)

White powder; IR (KBr) cm^{-1} ; 2916.52, 2848.58, 1736.75, 1462.85, 719.38; ^1H NMR (500 MHz, CDCl_3 , δ , ppm, J/Hz); 2.34 (2 H, t, $J=4.1$, H2), 2.10 (3 H, s-H4), 1.50 (2 H, m-H3), 1.20 (2 H, m-H5), 1.20 (2 H, m-H6), 1.20 (2 H, m-H7), 1.20 (2 H, m-H8) and 1.20 (2 H, m-H9), 0.89 (3 H, $J=3.8$, t-H10); ^{13}C NMR (500 MHz, CDCl_3 , δ , ppm); 207.3 (C1), 43.9 (C2), 31.9 (C4), 30.9 (C5), 32.9 (C3), 29.6 (C6), 29.3 (C7), 25.7 (C8), 22.7 (C9), and 14.3 (C10).

Decan-2-one (2) was obtained from dichloromethane crude extract as a white powder. Methylene/methyl

Bioscreening

The dichloromethane extract produced the highest antiplasmodial activity against *Plasmodium falciparum* 3D7 strain followed by MeOH, Hex, EtOAc and Aqueous (AEX), with IC_{50} values of 0.1177, 1.189, 4.850, 4.856, and 10.24 $\mu\text{g/mL}$ respectively whereas artesunate (positive control) was very active as expected (IC_{50} : 3.629×10^{-3} $\mu\text{g/mL}$) as represented in Table 4. The crude extracts can be identified as: highly active, promisingly active, moderately active; weakly active and inactive with IC_{50} values of 0–5, 5–15, 15–50, 50–100 and greater than 100 $\mu\text{g/mL}$ respectively [60, 61]. Therefore, Hex, MeOH, DCM, and EtOAc are considered to have high antiplasmodial activity while the aqueous extract has promising activity. Differences in the activities of the extracts could have resulted from varying amounts of phytochemicals in each extract.

Table 4 IC₅₀ results for the extracts and the isolated compounds

Name	Test samples	IC ₅₀ values (µg/mL)		
		<i>P. falciparum</i> 3D7 strain	Clinical field isolate (KOM_234)	Clinical field isolate (KDH_204)
Extract	Hex	4.850 ± 0.120	-	-
	DCM	0.1177 ± 0.097	-	-
	EtOAc	4.856 ± 0.261	-	-
	MeOH	1.189 ± 0.201	5.30 ± 1.10	5.06 ± 2.23
	Aqueous	10.24 ± 0.137	-	-
Compounds	Allantoin	-	2.29 ± 0.98 [14 µM] [*]	10.94 ± 1.51 [69 µM] [*]
	Decan-2-one	27.44 ± 3.316 (176 µM) [*]	-	-
Reference standards	CQ	-	0.0074 ± 2.3 × 10 ⁻³	0.0037 ± 9.4 × 10 ⁻⁴
	MQ	-	0.085 ± 4.1 × 10 ⁻⁴	0.066 ± 3.2 × 10 ⁻²
	Artesunate	3.629 × 10 ⁻³ ± 1.041 × 10 ⁻³	-	-

CQ Chloroquine, MQ Mefloquine, -: Not done.

The results are presented as the means ± SDs (n = 4). *IC₅₀ values are presented in µM

The methanol extract had promising antiplasmodial activity against the clinical field isolates KOM_204 and KDH_234 with IC₅₀ values of 5.3 and 5.06 µg/mL respectively. However, it was more active against the clinical field isolate KOM_234 than against KDH_204. The difference in the antiplasmodial activity of the methanol extract on KOM_234 and KDH_204 is very small which shows that the compounds found in the extract could exhibit some similar activity against both clinical field isolates.

The levels of activity of compounds 1 and 2 were classified accordingly [60, 61]. Compared with KDH_204, which has moderate activity (IC₅₀: 69 µM), compound 1 is highly active (IC₅₀ of 2.29 µg/mL) against the field isolate (KOM_234). Additionally, this compound showed greater activity against the clinical field isolate KOM_234 than its methanol extract (IC₅₀ of 5.30 ± 1.10 µg/mL), as expected. However, it was less active than its methanol extract (IC₅₀ 5.06 ± 2.23 µg/mL) against the field clinical isolate KOM_234 [Table 4]. These outcomes could be explored further for antiplasmodial activity. Previous studies have shown that batesin, a derivative of Allantoin isolated from *Cordia batesii*, has high antiplasmodial activity [62] and that allantoin isolated from *C. viscosa* has antibacterial activity [63].

Compound 2 exhibited low activity (176 µM) against the *Plasmodium falciparum* 3D7 strain and was much less active than its extract, i.e., the DCM extract (IC₅₀: 0.1177 µg/mL) [Table 4]. It is possible that the active compound concentration was lower in the extract or that the activity was influenced by other compounds present in the extract. In other studies, the good mosquito repellency activity of Decan-2-one with other aliphatic ketones was established [64], and essential oil from *Houttuynia cordata*, which contains 4.99% of 2-decanone, has been found to be very active against cancer [57]. A volatile component from fruit extract (which contains

2-decanone) exhibited antioxidant and anti-inflammatory effects [65].

Allantoin and decane-2-one demonstrates some antiplasmodial potential, no previous studies has shown the compounds as a frontline antimalarial. Future research should explore their mechanisms, synergistic combinations, and efficacy in animal/human trials.

Molecular docking

The bioassays, revealed that the *P. falciparum* strains used were chloroquine-sensitive. Hence chloroquine was used as a reference compound for docking. PfLDH is considered an important enzyme for the survival of *P. falciparum* as it is involved in glycolysis, converting pyruvate to lactate with concomitant energy release. PfLDH is also structurally and functionally different from its human counterpart [66]. All these factors make it a suitable target for drug development. Studies have shown the possible allosteric inhibition of the druggable substrate binding site. Chloroquine is known to competitively inhibit NADH at the cofactor binding site [67]. The compounds were thus docked into two different sites on PfLDH: the enzyme active site represented by 1U5A and the cofactor binding site represented by 1CET. Figure 2 shows the model of the protein with the two sites as well as the ligand interactions observed for the best scoring ligand, 10 (2-myristoyl pantetheine). The validation of the docking protocols can be verified from Figure S7 (Supplementary data) which shows RMSD values of 0.5030 and 1.3713 for 1U5A and 1CET respectively. An RMSD value of < 2 is generally considered adequate [35].

Table S2 (supplementary data), shows that better docking scores were obtained for the active site even for chloroquine which has been shown to bind near or in the cofactor binding site. This was in line with the study by Shamsuddin et al. where the ligands preferably bound to the active site [35].

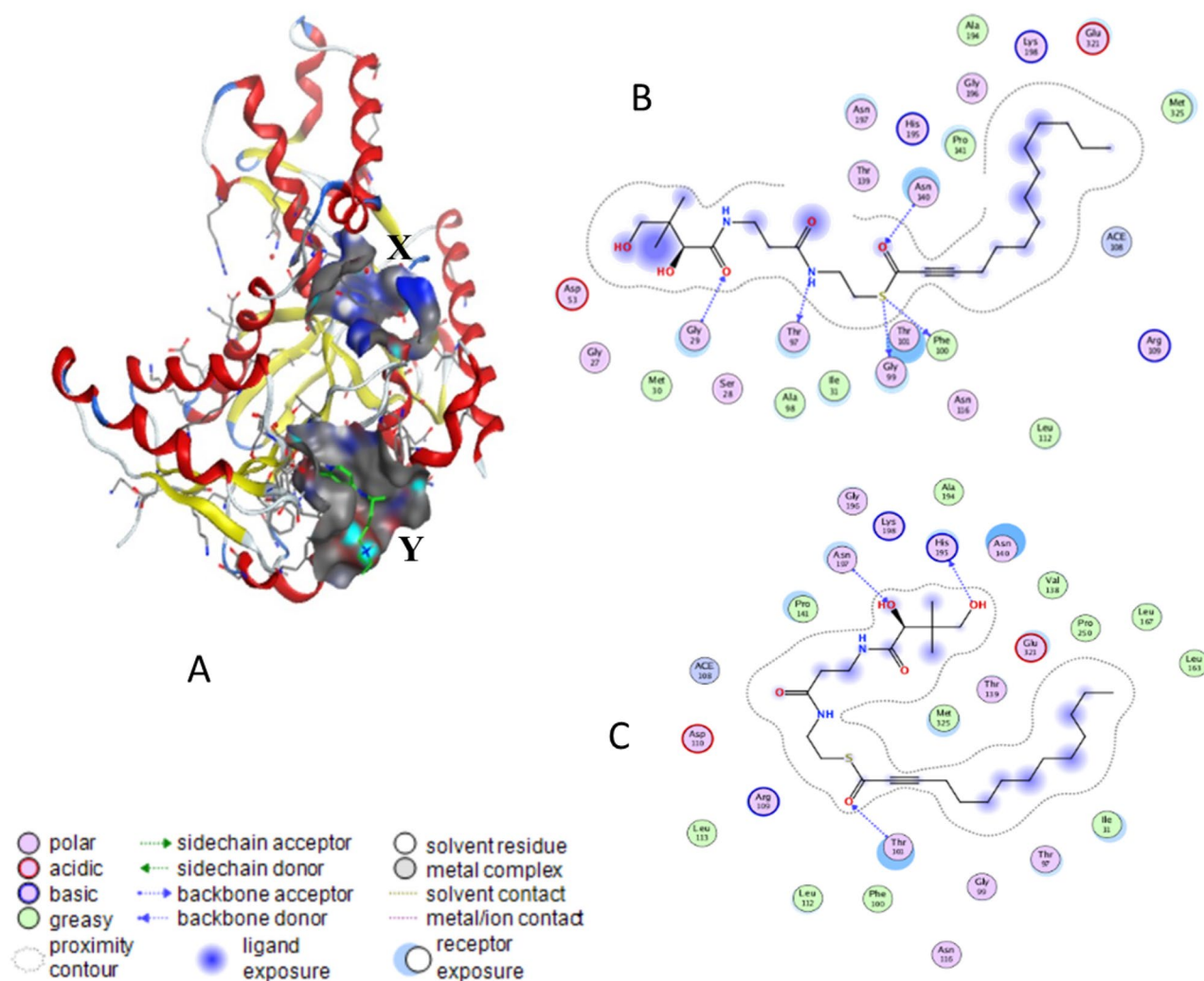


Fig. 2 **A:** 3D-model of PflDH showing the active binding site (X) and the cofactor binding site (Y); 2D rendering of **B:** ligand interactions between 10 and the PflDH active site and **C:** ligand interactions between 10 and the PflDH cofactor binding site

Six compounds performed better than the reference compound chloroquine namely; compounds 3, 6, 8, 10, 12 and 15. The isolated compounds Allantoin and decan-2-one were among the compounds with the lowest docking scores. This is corroborated by their low antiparasitic activity. Compound 10 had the best predicted docking score for both binding sites; $-8.5190\text{\AA}$ and $-7.323\text{\AA}$ for the enzyme active and co-factor binding sites respectively (Table S2).

The ligand interactions observed in the active site (Fig. 2) involve bonding with His 195 and Asn 197 (donors) which are considered important residues for enzyme activity [35]. An additional interaction with Thr 101 (donor) was also observed. The observed hydrophobic and electrostatic interaction were also consistent with the literature and can be seen in the supplementary data.

In the case of the cofactor site, hydrogen bonding was observed with Met 30 (backbone donor) and Asp 53 (side

chain acceptor). The interactions observed were consistent with those reported for NADP [68]. Therefore, CPD 10 may act via more than the mechanism of inhibition tested in this study. Further *in silico* experiments such as molecular dynamics, to elucidate this phenomenon.

Conclusion

The reasonable antiparasitic activity exhibited by *C. opulifolium* leaf extracts is attributable to the presence of a combination of phytochemicals including alkaloids, steroids, coumarins, phlobatannins, reducing sugars, flavonoids, tannins, saponins, phenolic compounds and terpenoids. Sixteen compounds were putatively identified in the dichloromethane extract of the plant. Allantoin and decan-2-one which were isolated from methanol and dichloromethane extracts respectively showed average antiparasitic activity. This was supported by the docking scores for both two of the compounds identified,

2-myristynoyl pantetheine had the best docking score (better than that of chloroquine). This compound will have to be further investigated in vitro and in silico to confirm its proposed antimalarial activity as it may prove to be a useful lead.

Abbreviations

¹³ C NMR	Carbon-13 nuclear magnetic resonance
¹ H NMR	Proton nuclear magnetic resonance
DMSO	Dimethyl sulfoxide
C. opulifolium	Chenopodium opulifolium
EtOAc	ethyl acetate
Hex	hexane
MeOH	methanol
MHz	Mega Hertz
NA	not applicable
SD	standard deviation
RPMI	Roswell Park Memorial Institute
UV	ultraviolet
IC ₅₀	50% inhibitory concentration, FTIR: Fourier transform infrared, GC-MS: gas chromatography-mass spectrometry

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12906-026-05361-y>.

Additional file 1: Figure S1. GCMS chromatogram of the DCM extract of C. opulifolium leaves. Figure S2. 1H NMR data (600 MHz, DMSO-d₆) of compound 1. Figure S3. 13C NMR data (600 MHz, DMSO-d₆) of compound 1. Figure S4. HMBC data (600 MHz, DMSO-d₆) of compound (1) Figure S5. 1H NMR data (500 MHz, CDCl₃) of compound (2) Figure S6. 13C NMR data (500 MHz, CDCl₃) of compound 2. Figure S7. 2-D models of docking outputs for the validation of the docking protocol. Table S1. Phytochemical screening of C. opulifolium organic and aqueous leaf extracts. Table S2. Docking scores (S) of the identified compounds within the active and cofactor binding sites of PfLDH.

Acknowledgements

My sincere thanks go to Makerere University for the research opportunity as well as the University of Ghana for the research affiliation. Moreover, am grateful to MAPRONANO for financial support. The manuscript was presented as a thesis according to the following link: <http://makir.mak.ac.ug/handle/10570/10273>.

Authors' contributions

ceptualization, plant collection and identification: JS, JN and LB; Methodology: JS, JN and LB; Endorsement: JN, LB and MAC; Spectroscopic and in silico analysis: JS, JN, LB, MAC, GAD and JNA; Antiplasmodial activity investigation: JS, HMA and LEA; Manuscript draft design: JS, JN and LB; Review and editing: JS, JN, LB, MAC and JNA; Supervision: JN, LB and MAC. Research management: JN, LB and MAC; Funding acquisition: JS, JN and LB. All the authors have read and consented to the published version of the manuscript.

Funding

This research was supported financially by Makerere University and MAPRONANO.

Data availability

Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 3 January 2025 / Accepted: 19 March 2026

Published online: 24 March 2026

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