Expression of the Fab enzymes (Fab I and Fab Z) from *Plasmodium falciparum* after exposure to *Artemisia afra* plant extracts and drugs screening

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**Abstract** The emergence and spread of drug resistance of the malaria parasite to the main treatment emphasize the need to develop new antimalarial drugs. In this context, the fatty acid biosynthesis (FAS_II) pathway of the malaria parasite is one of the ideal targets due to its crucial role in parasite survival. In this study, we report the expression and the affinity binding of Fab_I and Fab_Z after exposure to the parasite with different extracts of the *Artemisia afra*. The parasites were exposed for 2 days to different extracts. Gene expression was done to determine the level of expression of the fab enzymes after treatments. A GCMS was run to determine the different compounds of the plant extracts, followed by a virtual screening between the fab enzymes and the active compounds using Pyrex. The results showed different expression patterns of the Fab enzymes. Fab_I expression was downregulated in the W2 and D6 strains by the ethanolic extract but was increased by Hexane and DCM extracts. A different expression pattern was observed for Fab_Z. It was all upregulated except in the D6 strain when exposed to the ethanolic and hexane extracts. Virtual screening showed an affinity with many compounds. Hits compounds with high binding energy were detected. 11alphaHydroxyprogesterone and Aspidospermidin-17-ol were found to have high binding energy with Fab_I respectively (−10.7 kcal/mol; −10.2 kcal/mol). Fab_Z shows also high affinity with 11alpha-Hydroxyprogesterone (−10 kcal/mol) and Thiourea (−8.4 kcal/mol). This study shows the potential of *A. afra* to be used as a new source of novel antimalarial compounds.

**Keywords** *Artemisia afra* · Fab_I · Fab_Z · Drugs screening · Gene expression

**Abbreviations**

| Abbreviation | Description |
|--------------|-------------|
| GCMS         | Gas Chromatography–Mass Spectrometry |
| Ct           | Cycle Threshold |
| PTFE         | Polytetrafluoroethylene |
| KEMRI        | Kenya Medical Research Institute |
| A            | Actin |
| FZ           | Fab_Z |

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Introduction

Malaria is still a big concern in the African health system due to its high number of death yearly. Africa is the most affected, where more than 90% of the death occur in those sub-Saharan countries. Today we are facing a big challenge concerning the spread of the resistance to main drugs wildly spread in South East Asia, a threat that can drive into Africa. New indigenous gene mutations of the *Plasmodium falciparum* parasite were discovered in Rwanda in 2020. The mutation concerns a new indigenous lineage, the *pfkelch13 R561H*, and can drive Artemisinin resistance (Uwimana et al. 2020). These raise the need to develop new drugs that can overcome that resistance. Researchers are increasingly focusing on using medicinal plants as a polytherapy that works better in overcoming parasite resistance. *Artemisia afra* is one of those promising medicinal plants with a high antimalarial effect and is not toxic in high dosage. This plant was growing wildly from the cape to the Eastern part of Africa and was used for decades by traditional healers. It is one of the best-known medicinal plants. The list of uses covers a wide range of ailments from coughs, colds, fever, loss of appetite, colic, headache, earache, and intestinal worms to malaria (Van Wyk et al. 1997; Gathirwa et al. 2007; Van der Kooy et al. 2008). In this project, different strains of *P. falciparum* parasite W2 (CQ resistant) and D6 (CQ sensitive) were exposed to different extracts of *Artemisia afra* collected from Burundi, to monitor the expression level of different genes Fab I and Fab Z that belong to the Fab enzymes, type II pathway implied in the fatty acid biosynthesis of malaria’s parasite. Those Fab enzymes are crucial for the parasite’s survival and spreading mostly during the liver stage, and are targets for the development of new drugs candidate. Studying their expression when exposing the parasites to the plant extracts can bring a lot of information about using those genes as a target for new drugs.

Materials and methods

*Artemisia afra* extraction

**Plant preparation**

Burundi at Rumonge South Burundi. The leaves were harvested before blooming and dried under shade before being sent to Nairobi in paper bags, then kept at room temperature until extraction.

**Extraction process**

Samples of dried and powdered aerial part of *A. afra* weighing each 125 gm were extracted with 600 mL of Ethanol (100%), Hexane (85%), DCM (99.9%) and in water (800 mL) in a flat bottomed flask and mixed on an orbital shaker. After gentle maceration for 48 h, the extracts were filtered through Whatman filter paper n°1. The filtrate was concentrated under reduced pressure using a rotary evaporator at 20 rpm and 40 °C bath temperature. Finally, concentrated extracts were collected in vials and placed in a water bath at 40 °C to evaporate the remaining solvents and stored at room temperature for complete dryness.

**Gene expression study for Fab_Z and Fab_I from *P. falciparum* after the exposition of the parasites to the crude extracts**

**Culture preparation and incubation**

Cultures of *Plasmodium falciparum* (W2 and D6) with a parasitaemia of 4% were incubated with *Artemisia afra* extracts collected from Burundi, e.g.: Burundi ethanolic, hexane and dichloromethane extract to run an inhibition test. *Artemisia afra* solution was made for all the extracts. 100 mg of extracts were dissolved in 200 ul of DMS; double distilled water was used to dilute the extracts. 20 ml of double distilled water was added to the final concentration of 5 ug/ul; then the solution was sterilized by filtering it through a microfilter of 0.45 um pore size.

- 6 ml of W2 cultures were incubated with 666 ul of *A. afra* extracts (DCM, ETOH, and Hexane plant extracts) at a final concentration of 0.5 µg/µl.
- 6 ml of W2 culture as negative control incubated with 666 ul CMS with DCMS (final DMSO concentration was 0.1%).
- 6 ml of D6 cultures were incubated with 666 ul of *A. afra* extracts (DCM, ETOH, and Hexane plant extracts) at a final concentration of 0.5 µg/µl.
- 6 ml of D6 culture as negative control were incubated with 666 ul CMS and DMSO (final concentration of DMSO was 0.1%).

The parasites were then kept for 2 days in the incubation room at 37 °C in a gas mixture, 3% CO2, 5% O2 and 92% N2. After the inhibition test, the parasite was kept at ~ 80 °C to conserve RNA integrity. The samples were used later for RNA extraction to run gene expression of FAB_Z and FAB_I in each sample.
RNA extraction

After the inhibition test was done, the total RNA for all samples (W2 and D6) was extracted by following the method of trizol reagent Invitrogen Company (http://tools.thermofisher.com/content/sfs/manuals/trizol_reagent.pdf).

The parasites were thawed in ice, the whole blood was centrifuged, and the supernatant was discarded. The pellet was lysed using 1 ml Trizol, then incubated at 37 °C for 5 min to ensure the complete deproteinization of nucleic acids. 200 ul of Chloroform was added, the tube was shaken vigorously by hand followed or using a vortex then centrifuged for 15 min at 12,000g at 4 °C. The upper aqueous phase was carefully removed and placed in a new tube. Isopropanol was added (to precipitate the RNA) then mixed and centrifuged at 12,000g at 4 °C for 15 mn. The supernatant was discarded and the pellet was suspended with 1 ml of 75% ethanol then vortex briefly and centrifuge for 10 mn at 7500g 4 °C. The supernatant was discarded and the pellet air-dried for 10 mn. The RNA was resuspended with 40 ul of RNAs-free water and incubated in a heat block for 10 min at 60 °C. The quantity and the quality of the RNA were determined respectively using Nano_Drop. The purity of the RNA sample was defined by the A260/A280 ratio. A ratio between 1.8 and 2.1 was indicative of highly purified RNA. The concentration of the extracted RNA was determined using the following equation:

\[
\text{RNA concentration (\mu g/\mu l) = \frac{(A260 \times 40 \times D)}{1,000}}
\]

where D = dilution factor.

cDNA synthesis

The RNA samples were normalized by adding RNA free water to get a concentration of 50 ug/ul of RNA for all samples; then the RNA was converted into cDNA by reverse transcription (RT). The oligo (DTs) primers were used for the reverse transcription. cDNA synthesis kit from Solis BioDyne was used for the reaction. The samples were incubated in a PCR machine at 50 °C for 45 mn to allow the reverse transcription reaction to take place and to be complete, then at 85 °C for 5 min to inactivate the enzyme and stop the reaction. The cDNA samples were then kept at − 20 °C awaiting further analysis.

FAB_Z FAB_I primers

Primers for FAB_Z and Fab_I were created using Prime 3 (http://bioinfo.ut.ee/primer3-0.4.0/) and ordered online. The primers were designed to avoid hairpins and self-annealing with a GC content around 40–50% and an annealing temperature of 60 °C.

A stock solution of 100 uM was prepared for each primer using PCR water buffer. 10 ul from the stock solution was added to 90 ul of PCR water to prepare a working solution of 10 uM.

- FAB_Z primer
  
  Forward TTTGCTGGAGTGGATGGAGT
  Reverse CGATAAGGCAAACGTCTTTCTGA
  Product size: 177 bp

- FAB_I primer
  
  Forward CGGGTGGGGTATTGCTAAAGAA
  Reverse AGAAGCGTCAAAAGGTTAGCA
  Product size: 178 bp

Conventional PCR for FAB_I and FAB_Z

The designed primers were tested during the conventional PCR, and their parameters were set up. 5 × FIREpol Master Mix ready to load with syber green dye was used for the conventional PCR. 4 ul of a master mix containing DNA polymerase, 5 × reaction buffer, 12.5 mM Mgcl2, 1 mM dNTPs, blue and yellow dye added into labelled PCR tubes 1 ul of primers solutions were added.

cDNA samples were thawed, and 2 ul were added to each PCR tube and topped up with water till 20 ul of the final volume. The sample was placed into the PCR thermocycler, and the run was set with different parameters until we found the right one that amplified better the genes. The following amplification program was finally used for the two primers (FAB_Z and FAB_I): Initial denaturation at 95 °C for 5 min and then 40 cycles of denaturation at 95 °C for 1 min, annealing 60 °C for 1 min, extension 72 °C for 1 min, followed by the final extension at 72 °C for 10 min, and then held at 4 °C. After gel electrophoresis was made to ensure that the primers got correctly amplified with those parameters set up.

Housekeeping gene for P. falciparum

To run gene expression of Fab enzymes: Fab_Z (Beta-hydroxyacyl-Acyl-carrier Protein Dehydratase) and Fab_I (Enoyl Acyl-Carrier-Protein Reductase), we needed a housekeeping gene as a reference. Actin was chosen as the housekeeper. Primers were made to amplify the actin gene during real-time PCR. Prime 3 was used to make the primers, and parameters were set to fit the same parameters as for Fab_z and Fab_I.

- Actin Primers:
A conventional PCR was run first to test the primers chosen for actin as amplified. The following parameters were used: Initial denaturation at 95 °C for 5 min and then 40 cycles of denaturation at 95 °C for 1 min, annealing 60 °C for 1 min, extension 72 °C for 1 min, followed by the final extension at 72 °C for 10 min, and then held at 4 °C. After gel electrophoresis was made to make sure that the primers got correctly amplified.

**Gel electrophoresis for PCR products**

After running the conventional PCR for the genes and the housekeeping gene, a gel was prepared for the PCR product. Agarose gel (2%) in 2×TBE buffer was prepared. Ethidium bromide was included in the gel. Eight μl of each amplification reaction was loaded onto the gel. The gel was visualized on a UV trans-illuminator and photographed (Fig. 1).

**Real-time PCR**

After a successful amplification for all the genes (Fab_I and Fab_Z) and the housekeeping gene (Actin), a Real-time PCR was run to study the expression of the genes Fab_I and Fab_Z when exposed to our active extracts *Artemisia afra*. 5×HOT FIREPol EvaGreen qPCR mix plus (No Rox) from Solis BioDyne was used for the quantitative real-time PCR. The qPCR master mix composition was Hot FIREpol DNA polymerase, 5×Evagreen qPCR buffer, 12.5 mM Mgcl₂, dNTPs, Evagreen dye, No ROX dye. 96 wells plates for real-time PCR were used to set the reaction. In each well, 10 μl of total volume solution was prepared, each containing 2 μl of master mix for qPCR, 0.5 μl for the reverse primers, 0.5 μl for the forward primers, 2 ul for cDNA and 5 μl of water. Each sample was tested in triplicate. Light Cycler 96 software was used to set the parameters for Real-time qPCR and to visualize the results.

**Virtual screening with *Artemisia afra* compounds found during GCMS and the Fab enzymes**

**Gas Chromatography–Mass Spectrometry (GCMS) analysis of Artemisia afra extracts**

*Artemisia afra* from Burundi was collected and extracted with three solvents: Dichloromethane, Ethanol, and Hexane. The extracts were dried using a rotary evaporator and kept in the oven at 37 C until complete evaporation of the solvents. Pure artemisinin crystal was used as a standard to test any presence of the compound in *Artemisia afra* extracts.

**Sample preparation** Samples were dissolved and diluted in suitable organic solvents i.e. dichloromethane and ethanol extracts were dissolved in methanol solvent, while hexane extract was dissolved in hexane solvent) and passed through carbon black to remove waxes and chlorophylls. The samples were filtered through 0.45 μm PTFE filters and then transferred to sample vials for GCMS analysis.

**GCMS method** A Shimadzu QP 2010-SE GCMS coupled with an auto-sampler was used for the analysis. Ultrapure He (99.999%) was used as the carrier gas at a flow rate of 1 ml/minute. A BPX5 non-polar capillary column, 30 m; 0.25 mm ID; 0.25 μm film thickness, was used for separation. The GC was programmed as follows: 60 °C; 10 °C/min to 250 °C (10 min). The total run-time was 30 min. Only 1 μl of the sample was injected. The injection was done in split mode, 10:1. The injection was done at 200 C. The interface temperature was set at 250 C. The EI ion source was set at 200 C. Mass analysis was done in full scan mode, 50–550 m/z. A solvent delay time of 2 min was used.

**Drug screening with PyRx**

After GCMS a library of compounds was prepared based on the GCMS results of *Artemisia afra* extracts from Burundi. The library was screened against two macromolecules (FAB_I FAB_Z) using Autodock Vina in PyRx 0.8 version (Trott and Olson 2010). PyRx is a Virtual Screening software for Computational Drug Discovery that can be used to screen libraries of compounds against potential drug targets. The software is open to access and is available online at [http://pyrx.sourceforge.net](http://pyrx.sourceforge.net)

**Preparation of the library of small molecules** The 3D structures of all the compounds from GCMS were searched from three chemical structure databases and downloaded. Three databases were used: ChemSpider ([http://www.chemspider.com/](http://www.chemspider.com/)), PubChem from NCBI ([https://pubchem.ncbi.nlm.nih.gov/search/](https://pubchem.ncbi.nlm.nih.gov/search/)), and ChEMBL ([https://www.ebi.ac.uk/chembl/](https://www.ebi.ac.uk/chembl/)) from the European Bioinformatics Insti-
tute (EBI). The small molecules (Compounds) were downloaded, visualized with Pymol and then saved in the same file in PDB format.

**3D structure of Plasmodium falciparum Fab enzymes: Fab Z and Fab I**  The 3D structure of the Fab enzymes: Fab_Z and Fab_I were downloaded from the RCSB protein database (PDB), (https://www.rcsb.org/). Fab_Z (PDB entry: 3AZA), (Maity et al. 2011); Fab_I (PDB entry: 3LT0) (Maity et al. 2010). The macromolecules were crystallized with their ligands. The macromolecules were opened in txt format with notepad then all the ligands were removed to free the interaction sites.

**Virtual screening with PyRx: Protocol**  PyRx has the two virtual screening software Autodock 4.0 and Autodock vina. Autodock Vina was used during the drug screening because AutoDock Vina significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4 and is faster (Trott and Olson 2010).

**Results**

**Gene expression results**

**Gel electrophoresis for PCR product**  After parasite RNA extraction with trizol from the samples, we got different concentrations of RNA for each sample after measuring with a nanodrop. Before making the cDNA, RNA concentration was normalized to get an equal amount in all samples (experimental and controls). A conventional PCR was then run to determine the amplification parameters for the primers, the Fab enzyme genes and the housekeeping gene (Actin). A gel was run to visualize the amplicons (Fig. 1).

The results showed that the primers had well amplified the Fab enzymes genes (FabI and FabZ) and the housekeeping gene. Many parameters were set to get the best amplification. The amplification was optimum with these parameters: Initial denaturation at 95 °C for 5 min and then 40 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, followed by the final extension at 72 °C for 10 min, and then held at 4 °C.

**Real-time amplification**  The same parameter for the conventional PCR was reported to do the real-time PCR for the gene expression study. The target genes were well amplified as shown in Fig. 2

In blue colour, we have the amplification for the housekeeping gene (Actin), in yellow the amplification for Fab_I, and in red colour the amplification for Fab_Z. In grey is the amplification of the negative control and for the empty wells (drawn down of the picture). Actin has started to be amplified, followed by Fab_I, then by Fab_Z. Actin was well amplified. He had the best fluorescent curve of 5.7, followed by Fab_I, which was also well amplified with a fluorescent curve of 5.7. Fab Z fluorescent curve was 4.8. Still, the amplification was less compared to the other genes.

**Gene expression for Fab I after exposition of the parasites to A. afra extracts**

The fold change expression of Fab_I and Fab_Z was calculated from the Ct (cycle threshold is defined as the number of cycles required for the fluorescent signal to cross the threshold) values generated during the real-time PCR, and the result is shown in Tables 1 and 2. The fold change for the untreated sample is 1, for the treated samples when the fold change is greater than 1 it means that the targeted gene is upregulated and when it is lower than 1 it means that the targeted gene is downregulated. The Fab_I gene from the W2 strain of P. falciparum was upregulated with the hexane extract and downregulated by the ethanolic and dichloromethane extracts of the plant. In the second strain for D6, we have a different path; Fab_I was upregulated by DCM extract and downregulated by hexane and ethanolic extracts. Fab_I appeared to be downregulated in the two strains by the ethanolic extract of Artemisia afra (see Table 1).

The logarithm 10 of the fold change for FabI in each treatment was calculated to make the histogram (Fig. 3). The histogram is centred in 0, the log tenfold change) of the untreated sample for D6 and W2. The up columns showed upregulation, and the down columns showed downregulation. Depending on the length of the column, we can see the level of expression on both sides when downregulated or upregulated. This figure shows Fab_I is upregulated in the hexane and DCM extract in the W2 and D6 strain. However, the upregulation appears to be three times higher in the D6 strain compared to the W2 strain. Fab I downregulation is higher when both strains expose the ethanolic extract.

**Gene expression for Fab_Z after exposition to A. afra extracts**

Gene expression was upregulated for Fab_Z in the W2 strain when exposed to the three different extracts. The expression was downregulated in the D6 strain, except for the DCM extract. The DCM extract upregulates Fab_Z in the two strains of P. falciparum.

The log10 of the fold difference was calculated (Fig. 4), and the histogram was made to show the expression of Fab_Z; this allowed a better view of the expression scheme.
for Fab_Z depending on the extracts used. The histogram is centred on 0. The up columns showed upregulation, and the down columns showed downregulation.

In this figure (Fig. 4), the downregulation of the enzyme Fab_Z by the ethanolic and the hexane extract in the D6 strain appear to be almost at the same level.

Table 1 Fold change expression for FabI after treatment with A. afra extracts

| EXTRACTS (A. afra) | Fold difference W2 | Fold difference D6 |
|--------------------|---------------------|---------------------|
| Untreated          | 1                   | 1                   |
| Hexane             | 1.46                | 0.47                |
| EToH               | 0.34                | 0.37                |
| DCM                | 0.74                | 3.27                |

Bold values indicate the fold is greater than 1

Table 2 Fold change expression for Fab_Z after treatment with A. afra extracts

| EXTRACTS (A. afra) | Fold difference W2 | Fold difference D6 |
|--------------------|---------------------|---------------------|
| Untreated          | 1                   | 1                   |
| Hexane             | 1.7                 | 0.3                 |
| EToH               | 2.4                 | 0.3                 |
| DCM                | 2.9                 | 4.7                 |

Bold values indicate the fold is greater than 1

For Fab_Z, the expression after treatment with A. afra extracts showed upregulation. The histogram for the untreated group is centred on 0. The up columns showed upregulation, and the down columns showed downregulation. In this figure (Fig. 4), the downregulation of the enzyme Fab_Z by the ethanolic and the hexane extract in the D6 strain appear to be almost at the same level.

GCMS results

After the GCMS, the results in Table 3 showed that the ethanolic and dichloromethane extract had a high content of Bicyclo [2.2.1] heptan-2-ol, 1,7,7-trimethyl- (1S-endo). The hexane extract had the same compounds but at a low amount compared to ethanolic and DCM extract. The major compound in the hexane extract is Eucalyptol (Area 55.52). This compound is also found in DCM extract at a lower amount (area 19.77) and in ethanolic extract of A. afra (5.59). Hexane extract has fewer compounds compared to the others.
Most of the compounds found in the ethanolic extract are also in methanolic extract.

**Drug screening with PyRx**

After GCMS of the ethanolic, hexane and dichloromethane extracts in total, 51 molecules were detected. The affinity binding of the molecules was tested against Fab_I and Fab_Z with vina. After drug screening, the binding energy for each molecule was determined and expressed in Kcal/mol see Table 4. The results showed that many compounds have a high affinity with the Fab enzymes.

The results showed some hit compounds for Fab_I and Fab_Z with high biding energy. 11alpha-Hydroxyprogesterone has high binding energy with FabI (−10.7 kcal/mol) and Fab Z (−10 kcal/mol). The second hit is Aspidospermidin, which also has high binding energy with FabI (10.2 kcal/mol). The third is Thiourea, which has a binding energy of −9 kcal/mol for FabI and −8.4 kcal/mol for Fab Z.

The binding site for Fab_I with 11 alpha-hydroxyprogesterone (hit1) and with Aspidospermidin (hit 2) was shown respectively in Figs. 5 and 6. The 3D structure of Fab_I was downloaded from PDB (Protein Data Bank), and the active sites were determined before docking. After the virtual screening, the ligands were found to bind inside the active site and were in interaction with many residues in the active site.

Residue with hydrophobic interaction: Ile 369, Phe 368, Tyr 267, Tyr 277, Ala 312, Gly 313, Ser 215, Pro 314, Tyr 111, Leu 265, Ala 320, Ala 319 and hydrogen bond with Ser 317.

Residue with hydrophobic interaction: Tyr 277, Tyr 267, Ala 320, Gly 313, Leu 315, Tyr 111, Ser 317, Gly 110, Met 281, Ala217, Ala 319, Ile 323, Lys 285, and Thr 266.

The binding site for Fab_Z with 11 alpha-hydroxyprogesterone (hit 1) and Thiourea,-(hit2) were shown respectively in Figs. 7 and 8. They are mostly hydrophobic interactions with the ligands and one hydrogen bind between Thiourea and the residue Tyr 100(G).

Residue with hydrophobic interaction: Phe 129(J), Pro 101(J), Pro 101(I), Tyr 100(I), Pro 128(I), Tyr 100 (I), Tyr 100(K), Asn 131 (L), Phe 129(J), Pro 128(J) and one hydrogen bond with Tyr 100(G).

**Discussion**

*Artemisia afra* is a big antimalarial plant, and it is as potent as *Artemisia annua*, where the main antimalarial drug, artemisinin is extracted. A lot of studies show his antimalarial activities. In vitro studies run by Gathirwa et al. in 2007 about methanolic and water extracts of *A. afra* collected from Kenya show strong IC50 respectively for MEOH 9.04 µg/ml (for D6); 3.98 µg/ml (for W2) and for the water extract 11.23 µg/ml (D6); 4.65 µg/ml (W2), during that study the in vivo antimalarial assay display also a huge chemosuppression which was greater than 70% (Gathirwa et al. 2007). More recent studies using different solvent (DCM, Water, Hexane, and EtOH) extracts of *a. afra* from Burundi had displayed also big in vitro and in vivo antimalarial activity against W2 and D6, with some extracts having an IC50 lesser than 3 µg/ml and an ED50 of 6.43 mg/ml (Kane et al. 2019a, b).

In this study, an inhibition test was run by incubating during 48 h the parasite cultures with *A. afra* extracts to study the expression level of the *P. falciparum* Fab enzymes (Fab_I and Fab_Z). The fab enzymes play an essential role in the fatty acid synthesis pathway (FAS II). The prodigious proliferative capacity of malarial parasites necessitates access to an abundant source of fatty acids (FAs). These carboxylic acid-linked acyl chains are required for the production of lipid species that are essential for parasite membrane and lipid body biogenesis (Palacpac et al. 2004). It has been shown that FAS II can be exploited for antimalarial drug discovery (Waller et al. 2003; Perozzo et al. 2002). In our results, Fab_Z (D6 strain) was downregulated by the ethanolic and hexane extract of *A. afra*. Fab_Z is the primary dehydratase that participates in the elongation cycle of saturated as well as unsaturated fatty acid biosynthesis (Sharma et al. 2003). Downregulation of Fab_Z can lead to parasite death due to its crucial role in the fatty acid de novo biosynthesis. Fab_I also play a crucial role during the liver stage of malaria infection (Yu et al. 2008). A downregulation of Fab_I can lead to a failure of the parasite to complete the liver stage by an inability to form intrahepatic merozoites that normally initiate blood-stage infection. The results showed a downregulation of Fab_I by the ethanolic and the DCM extracts of *Artemisia afra* for the W2 strain. In the D6 strain, the gene is also downregulated by the hexane and...
Table 3  Main compounds detected by GCMS from dichloromethane extract, hexane and ethanolic extract of *A. afra* and result for GCMS for Artemisinin standard

| Standard/Extracts | Name of the compounds | Retention time (s) | Area % |
|-------------------|-----------------------|-------------------|--------|
| *A. afra* DCM     | 3,3,6-Trimethyl-1,4-heptadien-6-ol | 5.964              | 0.08   |
|                   | **Eucalyptol**          | **6.751**          | **19.77** |
|                   | 1,5-Heptadien-4-one, 3,3,6-trimethyl- | 7.054              | 6.08   |
|                   | 1-Methyl-4-(1-methylethynyl)-, cis- | 7.377              | 0.68   |
|                   | Linalool                | 7.76              | 1.06   |
|                   | 5-Isopropyl-2-methylbicyclo[3.1.0]hexan-2-ol # | 7.909              | 1.79   |
|                   | Camphor                | 8.794             | 1.29   |
|                   | Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-end)- | 9.176              | 31.87  |
|                   | Hexane, 1,6-dimethoxy- | 9.235             | 0.46   |
|                   | Cyclohexanol, 2-methyl-3-(1-methylethynyl)-, (1alpha.,2alpha.,3alpha.-) | 10.013             | 0.81   |
|                   | 1-Acetoxy-p-menth-3-one | 10.476             | 0.65   |
|                   | 1,7-Octadiene-3,6-diol, 2,6-dimethyl- | 10.578             | 0.56   |
|                   | Bornyl acetate         | 10.796            | 2.63   |
|                   | (1S,5S,6R)-6-Methyl-2-methylene-6-(4-methylpent-3-en-1-yl)bicyclo[3.1.1]heptane | 13.048             | 1.01   |
|                   | Methyl (1R,2R,8aS)-2-(methoxycarbonyl)-2-hydroxy-5,5,8a-trimethyl-trans-decalin-1-acetate | 14.151             | 0.52   |
|                   | 2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl- | 14.602             | 0.52   |
|                   | 2,2,4-Trimethyl-1,3-pentanediol diisobutyrate | 14.854             | 0.71   |
|                   | Caryophyllene oxide    | 15.165            | 1.08   |
|                   | .alpha.-Cadinol         | 15.867            | 5.27   |
|                   | (1S,4aS,7R,8aS)-1,4a-Dimethyl-7-((prop-1-en-2-yl)decahydrophthalen-1-ol | 16.258             | 2.86   |
|                   | Pulegone               | 17.514            | 0.96   |
|                   | 2-Pentadecanone, 6,10,14-trimethyl- | 17.837             | 1.64   |
|                   | 6-C14H26               | 20.551            | 0.42   |
|                   | Ethyl 9,12,15-octadecatrienoate | 20.636             | 0.52   |
|                   | Phytol                | 20.704            | 5.28   |
|                   | Geranyl ethyl ether 2  | 21.142            | 1.99   |
|                   | 11alpha-Hydroxyprogesterone | 21.325             | 2.31   |
|                   | 11alpha-Hydroxyprogesterone | 22.075             | 3.77   |
|                   | 1-Aminocyclopentane-carboxylic acid, N-(but-3 yn-1-yl oxy carbonyl)-, but-3-yn-1-yl ester | 22.97              | 1.59   |
| *A. afra* Hexane  | Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylen e-, (1S)- | 5.4               | 2.68   |
|                   | **Eucalyptol**          | **6.745**          | **55.52** |
|                   | 1,5-Heptadien-4-one, 3,3,6-trimethyl- | 7.048              | 24.19  |
|                   | Undecane               | 7.621             | 2.45   |
|                   | Dodecane              | 9.231             | 3.62   |
|                   | Acetic acid, 1,7,7-trimethyl-bicyclo[2.2.1]hept-2-yl ester | 10.783             | 3.72   |
|                   | .alpha.-Copaene       | 12.167            | 2.95   |
|                   | Tetradecane           | 12.221            | 1.24   |
|                   | Caryophyllene         | 12.87             | 2.42   |
|                   | 4a,8-Dimethyl-2-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,7-octahydrophthalene | 13.764             | 1.21   |
| *A. afra* Ethanolic Extract | 3,3,6-Trimethyl-1,4-heptadien-6-ol | 5.967             | 0.45   |
|                   | Eucalyptol            | 6.75              | 5.58   |
|                   | 1,5-Heptadien-4-one, 3,3,6-trimethyl- | 7.054              | 3.23   |
|                   | 2,7-Dimethyl-2,6-octadien-4-ol | 7.417             | 1.01   |
|                   | Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)- | 8.796             | 1.02   |
|                   | **Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-end)-** | **9.173** | **30.12** |
|                   | 3,7-Octadiene-2,6-diol, 2,6-dimethyl- | 9.227             | 0.69   |
|                   | 1,7-Octadiene-3,6-diol, 2,6-dimethyl- | 10.573             | 1.11   |
|                   | Bornyl acetate        | 10.794            | 0.96   |
|                   | 3-Cyclohexene-1-methanol, 2-hydroxy-,.alpha.,.alpha.,4-trimethyl- | 11.932            | 1.5    |
ethanolic extracts; this indicates that *Artemisia afra* could act as a drug with a prophylactic effect that can be able to stop the parasite in the earlier liver stage before the spread into the bloodstream. A study run in 2022 by Ashraf et al. came to strengthen this statement, where an in vitro exposition of *Artemisia afra* tea extract to the pre-erythrocytic (hepatic) stages of *Plasmodium* species (*P. berghei* and *P. falciparum*) led to a significant dose-dependent reduction in the size and number of hepatic schizonts. *Artemisia afra* (4 g/l) completely cleared parasites from the cultures after 4 days of treatment for *P. falciparum* and after 2 days of treatment for *P. berghei* with no cytotoxic effect (Ashraf et al. 2022).

**GCMS of Artemisia afra extracts**

GCMS was run in all three extracts: ethanolic, hexane and dichloromethane of *Artemisia afra* leaves from Burundi. 10 compounds were found in hexane extract; the major compound was Eucalyptol (55.5%). Cineole or Eucalyptol has mucolytic, bronchodilating and anti-inflammatory properties. It reduces the exacerbation rate in patients suffering from COPD (chronic obstructive pulmonary disease) and ameliorates symptoms in patients suffering from asthma and rhinosinusitis (Fischer and Dethlefsen 2013). It acts as a bronchodilator, can reduce inflammation, and can be used as a tonic to promote relaxation and reduce exhaustion (http://acupuncturetoday.com/herbcentral/borneol.php). In the methanolic extract, the major compound was also Borneol (Bicyclo [2.2.1] heptan-2-ol, 1, 7, 7-trimethyl-, (1S-endo) (40.6%). The majority of the compounds found in the ethanolic were also found in the dichloromethane and some in the hexane extract. The same major compound was found by Mwangi et al. (1995) when analyzing the essential oil from *Artemisia afra* by GCMS. The major constituents were 1, 8-cineole (67.4%), terpinen-4-ol (6.5%) and borneol (5.1%). The medicinal effect attributed to that plant can be because of the presence of all those active compounds. The first line against malaria is artemisinin and derivatives extracted from *Artemisia annua*. Artemisinin was not detected in all three extracts of *Artemisia afra*, so this means that the antimalarial activity lay on the other actives compounds of that plant.

**Affinity binding with FabI and Fab Z**

A library was created based on the compounds found during GCMS and was screened against the two fab enzymes (Fab I and FabZ) to test virtually their potential interactions using Autodock Vina with PyRx.

Autodock Vina is a docking software that aims to predict the ligand–protein complex structure interaction by exploring the conformational space of the ligands within the binding site of the protein. A scoring function is then utilized to approximate the free energy of binding between the protein and the ligand in each docking pose (Lionta et al. 2014).

After the virtual screening, all compounds were found to display a binding affinity that varies from high to low binding energies with the Fab enzymes, this may indicate

**Table 3** (continued)

| Standard/Extracts Name of the compounds | Retention time (s) | Area % |
|----------------------------------------|-------------------|-------|
| (1S,2S,4S)-Trihydroxy-p-menthane       | 13.964            | 1.99  |
| 4-O-Methylmannose                      | 14.289            | 6.76  |
| .alpha.-Cadinol                        | 15.863            | 1.73  |
| .alpha.-Methyl mannofuranoside         | 16.132            | 18.51 |
| (1S,4aS,7R,8aS)-1,4a-Dimethyl-7-(prop-1-en-2-yl)decahydronaphthalen-1-ol | 16.253 | 2.06 |
| Longifolentaldehyde                    | 17.907            | 3.05  |
| Pentadecanoic acid                     | 19.154            | 0.75  |
| Longifolentaldehyde                    | 19.276            | 1.56  |
| Phytol                                 | 20.703            | 1.67  |
| Corymbolone                            | 21.316            | 3.57  |
| 5.beta.-Androstan-3.alpha.,11.alpha.,17.beta.-triole | 22.07 | 1.58 |
| 2-((2R,4aR,8aS)-4a-Methyl-8-methylene  | 24.025            | 3.35  |
| 1H-1,3a-Ethanopentalen-5(4H)-one, 2,3-dihydro- | 24.124 | 3.18 |
| Thiourea, 1-(adamentane-1-carbonyl)-3-cyclohexyl- | 26.574 | 4.57 |

Bold values indicate the most abundant compounds after GCMS
| Ligands                                                                 | Binding energy (Kcal/mol) (FabI) | Binding energy (Kcal/mol) (FabZ) |
|------------------------------------------------------------------------|----------------------------------|----------------------------------|
| (1S, 2S,4S)-Trihydroxy-p-menthane                                       | −6.4                             | −6.1                             |
| .alfa.-Copaene                                                          | −7.9                             | −7.1                             |
| .alpha.-Cadinol                                                         | −8.1                             | −7.4                             |
| .alpha.-Methyl_mannofuranoside                                          | −5.7                             | −5.3                             |
| 1_6-Dimethoxyhexane                                                    | −4.7                             | −4.9                             |
| 1_7-Octadiene-3_6-diol_2_6-dimethyl-                                   | −5.7                             | −6.6                             |
| 1-Acetoxy-p-menth-3-one                                                | −6.9                             | −6.9                             |
| 1-Aminocyclopentanecarboxylic_acid_N-but-3-yn-1-yloxy-carbonyl-but-3-yn-1-yl_est   | −7.1                             | −4.9                             |
| 1H-1,3a-Ethanopentalen-5(4H)-one_23-dihydro-                           | −6.7                             | −6.5                             |
| 2-(2R,4aR,8aS)-4a-Methyl-8-methylene-dicahydro-naphthalen-2-yl-prop-2-en-1-ol | −7.9                             | −6.1                             |
| 2_2_4-Trimethyl-1_3-pentanediol_dissobutyrate                           | −6.8                             | −6.6                             |
| 2_4H_-Benzofuranone_5_6_7_7a-tetrahydro-4_4_7a-trimethyl-              | −7.2                             | −6.2                             |
| 2_7-Dimethyl-2_6-octadien-4-ol                                         | −6.3                             | −6.6                             |
| 2-Pentadecanone_6_10_14-trimethyl-                                     | −6.2                             | −5.5                             |
| 3_7-Octadiene-2_6-diol_2_6-dimethyl-                                   | −6.1                             | −5.2                             |
| 3-Cyclohexene-1-methanol_2-hydroxy-.alpha._.alpha._4-trimethyl-       | −7.0                             | −5.8                             |
| 3-Iso-propenyl-2-methylcyclohexanol                                    | −6.7                             | −6.9                             |
| 4a_8-Dimethyl-2-prop-1-en-2-yl_1_2_3_4_4a_5_6_7-octahydronaphthalene  | −8.0                             | −5.6                             |
| 4-O-Methylmannose                                                      | −6.2                             | −4.8                             |
| 5.beta.-Androstan-3-alphalalpha.17.beta.-triol                           | −9.7                             | −7.6                             |
| 6-C14H26_tetradeicyne                                                 | −5.8                             | −6.3                             |
| Acetic_acid_1_7_7-trimethyl-bicyclo_2_2_1_hept-2-yl_est                | −6.9                             | −5.9                             |
| Aspidospermidin-17-ol, 1-acetyl-19_21-epoxy-15_16-dimethoxy            | −10.2                            | −7.6                             |
| Bergamotene                                                            | −7.5                             | −8.0                             |
| Bicyclo_2_2_1_heptan-2-one_1_7_7-trimethyl_-1S_-                       | −6.3                             | −6.7                             |
| Bicyclo_221_3d                                                         | −6.2                             | −6.6                             |
| Borneol                                                                | −6.5                             | −6.7                             |
| Bornyl_acetate                                                         | −6.9                             | −5.9                             |
| Camphor                                                                | −6.3                             | −6.7                             |
| Caryophyllene                                                          | −8.0                             | −7.3                             |
| Caryophyllene_oxide                                                    | −8.3                             | −7.0                             |
| Caryomolone                                                            | −8.6                             | −7.3                             |
| Dodecane                                                               | −5.0                             | −5.8                             |
| Ethyl_9_12_15-octadecatrienoate                                        | −6.6                             | −5.7                             |
| Eucalyptol                                                              | −6.4                             | −5.9                             |
| Geranyl_ethyl_ether_2                                                  | −5.6                             | −5.9                             |
| Intermederol                                                           | −7.8                             | −7.0                             |
| Isoartemisia_ketone                                                    | −6.0                             | −6.0                             |
| Linalool                                                                | −5.8                             | −6.2                             |
| Longifolonaldehyde                                                     | −7.9                             | −7.1                             |
| Methyl_1R_2R_8aS_2-methoxy-carbonyl_2-hydroxy-5_5_8a-trimethyl-trans-decalin-1-acetate | −8.3                             | −6.2                             |
| Pentadecanonic_acid                                                    | −5.6                             | −4.8                             |
| Phytol                                                                | −6.4                             | −7.2                             |
| 11alpha-Hydroxyprogesterone                                            | −10.7                            | −10.0                            |
| Pulegone                                                               | −7.8                             | −5.7                             |
| Tetradecane                                                            | −5.2                             | −5.5                             |
| Thiourea, 1-(adamantane-1-propyl)-3-cyclohexyl                          | −9.0                             | −8.4                             |
| Thujaanol                                                              | −6.3                             | −6.3                             |
| Trans-terpineol                                                        | −6.8                             | −6.2                             |
that all active compounds of *Artemisia afra* may play a significative role by interacting with the *P. falciparum* Fab enzymes, which has a significant role in parasite survival during malaria infection. A comparison of the different binding energies for all molecules has shown up some dock hits. It was found that 11-alpha-Hydroxyprogesterone, Aspidospermidin-17-ol, 1-acetyl-19, 21-epoxy-15, 16-dimethoxy, and Thiourea, 1-(adamantane-1-carbonyl)-3-cyclohexyl were the efficient binders, with high binding energy. There is no literature about the pharmacological

**Table 4 (continued)**

| Ligands        | Binding energy (Kcal/mol) (FabI) | Binding energy (Kcal/mol) (FabZ) |
|----------------|----------------------------------|----------------------------------|
| Undecane       | −4.7                             | −5.8                             |

Bold values indicate the highest binding energy that identifies the hit compounds.

**Fig. 5** Interaction site for FabI (chain B) and hit N°1 (Hydroxyprogesterone)

**Fig. 6** Interaction site for FabI (chain B) and hit N°2 (Aspidospermidin)
Aspidospermidin- and Thiourea, 1-(adamantane-1-carbonyl)-3-cyclohexyl. 11alpha-Hydroxyprogesterone has recently been patented for treating skin diseases, particularly psoriasis. It is an important pharmaceutical compound with anti-androgenic and blood-pressure-regulating activity (Nguyen et al. 2012). Those active compounds were also found to interact with most of the residue of the active side of Fab I. However, an in vitro test is necessary to confirm their antimalarial activity.

**Conclusion**

This study shows the high potential of using *Artemisia afra* as a source in the search for new antimalarial drugs. The FAS II pathway constitutes a prime target for developing prophylactic and curative drugs.

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**Authors contribution** Dr. NJ: corresponding author, study design, performs experimentations, data analysis, and article writing. Dr. MCK: study design, data analysis, writings. Dr. JKN: study design, writings. Pr. AH: study design, writings. Dr. MD: study design, writing. Dr. FK: Bring facilities to perform experiments.

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### References

Ashraf K, Tajeri S, Arnold C-S, Amanzougaghene N, Franetich J-F, Vantaux A, Soulard V, Bordessoules M, Cazals G, Bousema T, van Gemert G-J, Le Grand R, Dereudre-Bosquet N, Barale J-C, Witkowski B, Snounou G, Duval R, Botté CY, Mazier D (2022) Artemisinin-independent inhibitory activity of *Artemisia* sp. infusions against different Plasmodium stages including relapse-causing hypnozoites. Life Sci Alliance 5(3):e202101237

Fischer J, Dethlefsen U (2013) Efficacy of cineole in patients suffering from acute bronchitis: a placebo-controlled double-blind trial. Cough 9:25. https://doi.org/10.1186/1745-9974-9-25

Gathirwa JW, Rukunga GM, Njagi ENM, Omar SA, Guantai AN, Muthaura CN, Mwitari PG, Kimani CW, Kirira PG, Tolo FM, Nduna TN, Ndige IO (2007) In vitro anti-plasmodial and in vivo antimalarial activity of some plants traditionally used for the treatment of malaria by the Meru community in Kenya. J Nat Med 61:261–268

Kane NF, Kyama MC, Nganga JK, Hassanali A, Diaoo M, Kimani FT (2019a) Acute toxicity effect of *Artemisia afra* plant extracts on the liver, kidney, spleen and in vivo antimalarial assay on Swiss Albino mice. Adv Biosci Bioeng 7:64. https://doi.org/10.11648/j.abb.20190704.12

Kane NF, Kyama MC, Nganga JK, Hassanali A, Diaoo M, Kimani FT (2019b) Comparison of phytochemical profiles and antimalarial activities of *Artemisia afra* plant collected from five countries in Africa. S Afr J Bot 125:126–133

Lioneta E, Spyrou G, Vassilatis DK, Cournia Z (2014) Structure-based virtual screening for drug discovery: principles, applications and recent advances. Curr Top Med Chem 14:1923–1938

Maity K et al (2010) X-ray crystallographic analysis of the complexes of enoyl acyl carrier protein reductase of *Plasmodium falciparum* with triclosan variants to elucidate the importance of different functional groups in enzyme inhibition. IUBMB Life 62:467–476

Maity K et al (2011) Structural basis for the functional and inhibitory mechanisms of β-hydroxyacyl-acetyl carrier protein dehydratase (FabZ) of *Plasmodium falciparum*. J Struct Biol 176:238–249

Mwangi JW, Achola KJ, Sinei KA (1995) Essential oil constituents of *Artemisia afra* Willd. J Essent Oil Res 7(1):97–99. https://doi.org/10.1080/104129095.1995.9698475

Nguyen KT, Virus C, Günnewich N, Hannemann F, Bernhardt R (2012) Changing the regioselectivity of a P450 from C15 to C11 hydroxylation of progesterone. ChemBioChem 13(8):1161–1166. https://doi.org/10.1002/cbic.201100811. ISSN1439-4227

Palacpac NM, Hiramine Y, Mi-ichi F, Torii M, Kita K, Hiramatsu R, Horii T, Mitamura T (2004) Developmental-stage-specific triacylglycerol biosynthesis, degradation and trafficking as lipid bodies in *Plasmodium falciparum*-infected erythrocytes. J Cell Sci 117:1469–1480

Perozzo R, Kuo M, Sidhu ABS, Valiyaveetil JT, Bittman R et al (2002) Structural elucidation of the specificity of the antibacterial agent triclosan for malarial enoyl acyl carrier protein reductase. J Biol Chem 277:13106–13114

Sharma SK, Kapoor M, Ramya TN, Kumar S et al (2003) Identification, characterization, and inhibition of *Plasmodium falciparum* β-hydroxyacyl-acetyl carrier protein dehydratase (FabZ). J Biol Chem. https://doi.org/10.1074/jbc.M304283200

Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. J Comput Chem 31:455–461

Uwimana A, Legrand E, Stokes BH et al (2020) Emergence and clonal expansion of in vitro artemisinin-resistant *Plasmodium falciparum kelch13* R561H mutant parasites in Rwanda. Nat Med. https://doi.org/10.1038/s41591-020-1005-2

Van der Kooy F, Verpoorte R, Marion Meyer JJ (2008) Metabolomic quality control of claimed antimalarial *Artemisia afra* herbal remedy and *A. fraxinella* and *A. annua* plant extracts. S Afr J Bot. https://doi.org/10.1016/j.sajb.2007.10.004

Van Wyk B, Van Oudshoorn B, Gericke N (1997) Medicinal plants of South Africa. Briza Publications, Pretoria

Waller RF, Ralph SA, Reed MB, Su V, Douglas JD et al (2003) A type II pathway for fatty acid biosynthesis presents drug targets in *Plasmodium falciparum*. Antimicrob Agents Chemother 47:297–301

Yu M, Kumar TRS, Nkrumah LJ et al (2008) The fatty acid biosynthesis enzyme FabI plays a key role in the development of liver-stage malarial parasites. Cell Host Microbe 4(6):567–578

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