

*In vitro* antiplasmodial activity and cytotoxicity of extracts and chromatographic fractions of twigs from *Pappea capensis* Eckl & Zeyh. (Sapindaceae)

J. Mcebisi Mabuza<sup>a,b\*</sup>, Marcel Kaiser<sup>c,d</sup>, M. Johanna Bapela<sup>a,b</sup>

<sup>a</sup>University of Pretoria, Department of Plant and Soil Sciences, Hatfield 0028, South Africa

<sup>b</sup>University of Pretoria Institute for Sustainable Malaria Control and Medical Research Council Collaborating Center for Malaria Research, South Africa

<sup>c</sup>Swiss Tropical and Public Health Institute, Allschwil, Switzerland

<sup>d</sup>University of Basel, Basel, Switzerland

\*Corresponding author. Email: mabuzamcebisi@gmail.com

## Highlights

- *Pappea capensis* twigs were extracted in both polar and non-polar solvents.
- Most dichloromethane extracts and fractions displayed higher antiplasmodial.
- Some methanol fractions had good antiplasmodial activity and need to be further explored.
- Antiplasmodial activity is tentatively attributed to lupeol, alpha amyryn and beta amyryn.
- Further isolation of unknown compounds is possible through dereplication approaches.

## **Abstract**

### *Ethnopharmacological relevance:*

The Vha-Venda people of South Africa use *Pappea capensis* Eckl & Zeyh. (Sapindaceae) twigs to treat malaria and its related symptoms.

### *Aim of study:*

The main aim of this study was to evaluate the antiplasmodial and cytotoxic activity of *P. capensis* extracts and chromatographic fractions. Spectroscopy analysis was conducted using <sup>1</sup>H NMR and GC-MS to tentatively identify the major classes of compounds and phytoconstituents that can be attributed to the observed antiplasmodial bioactivity.

### *Materials and methods:*

*Pappea capensis* twigs were dried and then ground to fine powder. A solvent mixture of dichloromethane: methanol: water (1:0.5:0.5, v/v) was used to extract. The polar extract was separated from the non-polar. The organic extract was dried under to yield a DCM (I = 60 g). The methanol in the aqueous extract was evaporated using a rotary vapour and the remaining water freeze dried to yield a water extract (II = 287 g). Extract I was further partitioned using a solvent mixture of DCM: MeOH (1:1, v/v), separated and concentrated under vacuum to yield dichloromethane (III = 40 g) and methanol (IV = 15 g) extracts. A water-based decoction (V = 10 g) was also prepared to establish the clinical relevance of the preparation administered by Vha-Venda people in South Africa. Extracts II, III and IV were further subjected to silica column chromatography, eluting with a series of different solvents with increasing polarity to yield a total of 25 fractions (A – Y). *In vitro* antiplasmodial tests on *Plasmodium falciparum* (NF54) and cytotoxicity screens on mammalian L-6 rat skeletal myoblast cells were performed on all extracts and fractions. Selectivity indices (SI) were also computed for all tested

extracts and fractions which were further subjected to  $^1\text{H}$  NMR spectroscopy and GC-MS analysis for the identification of the major classes of compounds present in the extracts.

*Results:*

From the assayed extracts, only extract I ( $\text{IC}_{50} = 2.93 \mu\text{g /ml}$ ;  $\text{SI} = 14$ ), III ( $\text{IC}_{50} = 2.59 \mu\text{g /ml}$ ;  $\text{SI} = 21$ ) and IV ( $\text{IC}_{50} = 3.56 \mu\text{g /ml}$ ;  $\text{SI} = 13$ ) demonstrated the best antiplasmodial activity and selectivity. Of all assayed fractions, only N ( $0.6 \mu\text{g /ml}$ ;  $\text{SI}=91$ ), D ( $0.85 \mu\text{g /ml}$ ;  $\text{SI} = 37$ ) and E ( $0.91 \mu\text{g /ml}$ ;  $\text{SI} = 30$ ) depicted the best antiplasmodial activity and selectivity. The  $^1\text{H}$  NMR analysis of the extracts and fractions identified the prominent class of constituents to be aliphatic based which was tentatively identified as terpenoids. When further GC-MS analysis was conducted, the presence of lupin-3-one, lupeol acetate,  $\alpha$ -amyrin, and  $\beta$ -amyrin phytoconstituents were tentatively confirmed. These constituents are triterpenoids with established antiplasmodial activity which can be tentatively attributed to the bioactivity observed in *P. capensis* twigs.

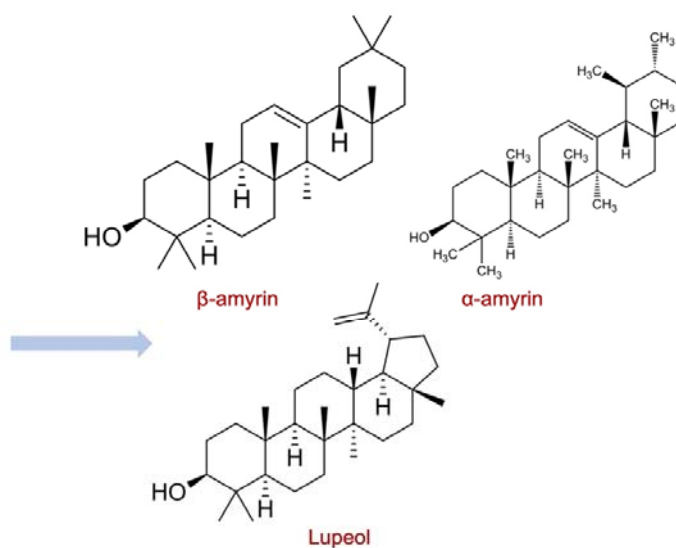
*Conclusion:*

The study validates the ethnomedicinal use of *P. capensis* for malaria treatment. It demonstrated the potential of discovering novel antiplasmodial constituents that could serve as drug hits through dereplication approaches where known compounds with established antimalarial activity can be bypassed to focus on the unknown.

## Graphical abstract



*Pappea capensis*



Antiplasmodial compounds

**Keywords:** Antiplasmodial activity; Sapindaceae; *Plasmodium falciparum*; *Pappea capensis*; Extracts; Fractions; Spectroscopy; South Africa

### 1. Introduction

*Plasmodium* wreaks havoc in malaria endemic sub-Saharan Africa (SSA) (WHO, 2020). The burden is worsened by the widespread effects of the SARS-CoV-2 virus, which presents symptoms like those of malaria (WHO, 2021). However, the reliance on medicinal plants to treat malaria continues to be prevalent in SSA. These plants have a long-standing history of use by indigenous people and a plethora of drugs have been delivered from active ingredients sourced from these botanicals. *Pappea capensis* Eckl. & Zeyh. is an indigenous plant species widely distributed in Africa (Robbertse et al., 2011). Taxonomically it is from the Sapindaceae family, and it is the only species in its genus. Its red edible fruits, a grey or pale stem with knobby branches are its distinct morphological characteristics (Mng'omba et al., 2008). It grows up to a height of about 3.9 m and survives in diverse ecological conditions

(Palmer and Pitman, 1972; Venter and Venter, 1996). In South Africa, it is found in the Northern Cape, the Karoo, Limpopo, Gauteng, and Kwa-Zulu Natal (Van Wyk and Gericke, 2000; Watt and Breyer-Brandwijk, 1962). The plant has genetically monoecious flowers, however, they display temporal dioecism due to varying environmental conditions like day length and temperature (Mng'omba et al., 2008).

Indigenous African people harvest *P. capensis* for different purposes. Its fleshy fruits produce vinegars, jellies and jams while the seeds are used to make edible oils and soaps (Mng'omba et al., 2008; Van Wyk and Gericke, 2000). Medicinally it is used to treat dysentery, eye infections, sexually transmitted infections, and can be used as an aphrodisiac (Chauke et al., 2015; Makule et al., 2014). The Maasai soak crushed bark in water and drink the residue as a tonic and malaria remedy (Karau et al., 2012; Watt and Breyer-Brandwijk, 1962). The Machakos and the Makueni communities in Kenya administer its root and bark infusion to treat chronic joint pains, while the Southern Ndebele tribes use that for sore eyes and purging cattle (Hutchings and Scott, 1996; Wambugu et al., 2011). Cold water infusion of the plant is traditionally used to treat venereal diseases and the Batswana use them as spiritual sprinkling charms (Hedberg and Staugård, 1989). The Ethiopian Gumuz people administer the bark decoction to treat malaria and tiredness (Asnake et al., 2016). Its water infusion boosts courage in warrior hunters and livestock headers, while in Venda the plant twigs are used to treat malaria (Bapela et al., 2014).

Modern cutting edge research approaches are continuously employed to solve many health problems that tend affect human health and wellbeing. This is quite evident in studies conducted on the antibacterial properties of DyBa<sub>2</sub>Fe<sub>3</sub>O<sub>7.988</sub>/DyFeO<sub>3</sub> and Co/Co<sub>3</sub>O<sub>4</sub> nanocomposites (Mahdi et al., 2022; Yousefi et al., 2021). Likewise,

different approaches have been utilized to understand the antiparasitic properties of *P. capensis*, even though the plant has not been extensively explored, there is on-going research to understand the medicinal properties of its different plant parts. The antiplasmodial activity of *P. capensis* is known to be possessed by the organic extracts of its leaves, roots, and twigs (Bapela et al., 2014; Koch et al., 2005; Mokoka et al., 2013). Bapela et al. (2014) extracted from the twigs using DCM: 50% methanol (1:1, v/v) and reported a significant antiplasmodial activity from the dichloromethane extract ( $IC_{50} = 5.47 \mu\text{g/ml}$ ). Mokoka et al. (2013) extracted from the roots and leaves using DCM: MeOH (1:1, v/v), and the antiplasmodial activity ranged from good to moderate ( $5.30 \mu\text{g/ml}$  and  $IC_{50} = 9.67 \mu\text{g/ml}$ , respectively). Koch et al. (2005) extracted from the inner bark of the plant using chloroform, and a low antiplasmodial activity was obtained  $IC_{50} \geq 10 \mu\text{g/ml}$ . Recently, Tajuddeen et al. (2021) have reported antiplasmodial activity in the MeOH extracts of *P. capensis* leaves. The main aim of this research was to study the antiplasmodial activity and cytotoxicity of *P. capensis* twigs to identify the different classes of compounds and phytoconstituents that can be attributed to the observed antiplasmodial activity. This would then set the stage for further dereplication of known constituents with established antiplasmodial activity from those unknown. Dereplication will then prioritize the isolation, characterization, identification, and biological assay screening of unknown phytoconstituents for antiplasmodial activity.

## **2. Materials and methods**

### *2.1.1. General*

The chemical profiles of extracts and fractions were analyzed on silica pre-coated thin layer chromatography (TLC) plates at room temperature (MERCK, silica gel 60 F254 0.2 mm thickness). Spots on the TLC were observed under UV light with

spectrum ranging from 254 nm – 366 nm. Silica gel was used as a stationary phase for column chromatography (CC). The various eluting solvents used were of analytical grade.

### 2.1.2. *Plant sample collection*

*Pappea capensis* twigs were collected from Sekhukhune, Limpopo, South Africa, and a voucher specimen (120331) was made and submitted in the H.G.W.J. Schweickerdt Herbarium (University of Pretoria). The plant twigs were dried at room temperature, ground to powder in an Ultracentrifugal Mill (ZM, 200, Retsch®, Germany).

### 2.1.3. *Extraction of plant samples*

About 2.5 kg of ground plant material was extracted using a solvent mixture of dichloromethane (DCM): methanol (MeOH): distilled water (dH<sub>2</sub>O) in a 1: 0.5:0.5 (v/v) ratio. The solution was filtered using Whatman's No. 1 filter paper. The extraction procedure was repeated thrice (Choi et al., 2004). The organic filtrate was evaporated in a vacuum yielding a DCM extract (I = 60 g). The MeOH in the aqueous extract was removed using a rotary vapour (Buchi, R – 200, Switzerland) at 40°C and the remaining water was freeze-dried (Virtis) to yield a water extract (II = 287 g). Extract I was further subjected to liquid-liquid separation using a solvent mixture of DCM: MeOH (1:1, v/v) to further ensure that all compounds with polarities overlapping either the polar or non-polar scale are kept separate. All non-polar compounds dissolved in the DCM layer while the polar ones dissolved in the MeOH layer. The two layers were then separated by filtration yielding extracts which were labelled III (40g) and IV (15 g) for DCM and MeOH respectively. Extract III was dried in a vacuum at 30°C and

extract IV was vaporized using a rotary vapor water bath (Buchi, R - 200, Switzerland) at 40°C. A decoction (V = 10 g) was prepared to simulate the traditional method used by indigenous people to establish the clinical relevance of the traditional use of *P. capensis*. The water-based decoction was prepared from 500 g of ground *P. capensis* twigs, boiled at 100°C for about 45 minutes to an hour. All polar and organic extracts were analysed independently.

#### 2.1.4. Column chromatography of *Pappea capensis* extracts

Extracts II (15 g), III (20 g), and IV (15 g) were subjected to silica CC. Extract II was eluted using ethyl acetate (EtOAc): MeOH, MeOH: distilled water (dH<sub>2</sub>O), at ratios of 1:10→10:1 for each solvent system. Extract III was chromatographed using hexane (H): DCM, DCM: EtOAc, EtOAc: MeOH, MeOH: dH<sub>2</sub>O at ratios of 1:10→10:1 for each solvent system. Extract IV was chromatographed eluting with DCM: EtOAc, EtOAc: MeOH, MeOH: dH<sub>2</sub>O at solvent ratios of 1:10→10:1 for each solvent system. All fractions collected were analysed using TLC and all fractions with chemical profile similarities were pooled together to yield a total of 25 fractions. Extract II produced eight fractions (R - Y), extract III produced nine fractions (A - I), and extract IV produced eight (J - Q).

#### 2.1.5. *In vitro* antiplasmodial assay

*In vitro* antiplasmodial screening of all extracts and fractions was conducted using the [<sup>3</sup>H] hypoxanthine incorporation assay method (Desjardins et al., 1979; Matile et al., 1990). The parasite strain used was *Plasmodium falciparum* (NF54) and chloroquine (Sigma C6628) as the standard drug (Ponnudurai et al., 1981). All samples were dissolved in dimethyl sulfoxide (DMSO) for parasite culturing and incubation at



RPMI 1640 in the absence of hypoxanthine. Some HEPES was added to the RPMI 1640 growth medium (5.94 g/l), NaHCO<sub>3</sub> (2.1 g/l), neomycin (100 U/ml), AlbumaxR (5 g/l) and human red blood cells A+ at 25% haematocrit (0.3% parasitaemia). Serial drug dilution of eleven 3-fold dilution steps covering the range of 100 – 0.002 ug/ml was prepared in 96 well plates, which were then incubated in a humidified environment at 37°C, 4% CO<sub>2</sub>, 3% O<sub>2</sub>, and 93% N<sub>2</sub>. A measure of 50 µl of [<sup>3</sup>H] hypoxanthine was added into each well after an hour and the plates for 24 hrs before the cells were harvested using a Betaplate<sup>TM</sup> cell harvester (Wallac). Red blood cells were transferred onto a glass fibre filter for rinsing with dH<sub>2</sub>O. A plastic foil with dry glass filters inserted with 10 ml of scintillation fluid was counted using the Betaplate<sup>TM</sup> liquid scintillation counter (Wallac). IC<sub>50</sub> values were calculated from sigmoidal inhibition curves by linear regression using GraphPad Prism 8.2.1 (Huber & Koella, 1993).

#### *2.1.6. Cytotoxicity assay of *Pappea capensis* extracts and fractions*

Cytotoxicity screening to determine the clinical safety of the assayed plant samples was done on rat skeletal cells. Microtiter plates (96-wells) were filled with 100 µl of RPMI 1640 medium supplemented with 1% L-glutamine (200 nM), 10% fetal bovine serum, and 4000 rat skeletal cell lines (Ahmed et al., 1994). Serial drug dilutions of eleven 3-fold dilution steps spanning the range of 100 – 0.002 µg/ml were prepared and after 70 hrs had lapsed, the incubated plates were observed under an inverted microscope. This was done to ensure the growth of the experimental control (podophyllotoxin) and the sterility of the conditions. Alamar Blue at a concentration of 10 µg/ml was then added to each well and the plates were further incubated for 2 hrs. After the incubation time had lapsed, the plates were read with the aid of a Spectramax Gemini XS microplate fluorometer (Molecular Devices Corporation) at an excitation

wavelength of 536 nm and an emission wavelength of 588 nm. The IC<sub>50</sub> values were computed with the aid of linear regression from the sigmoidal curves using the SoftmaxPro software (Molecular Devices Corporation) (Huber & Koella, 1993).

#### 2.1.7. *<sup>1</sup>H NMR analysis of Papeea capensis extracts and fractions*

<sup>1</sup>H NMR analysis was conducted to identify the different classes of compounds in the different extracts. Each extract was reconstituted in deuterated dimethyl sulfoxide (DMSO-d<sub>6</sub>) to a concentration of 15mg/ml. About 700 µl was transferred into a 5mm NMR tube (Wilmad, Sigma-Aldrich). Extracts were measured using the Varian 200 MHz <sup>1</sup>H NMR spectrometer and fractions using the 400 MHz spectrometer ran at a temperature of 25 °C, respectively. The spectral width of each spectrum was 14 ppm, and the acquired scans were 512 per sample. The obtained spectral data were referenced to the DMSO-d<sub>6</sub> solvent peak (2.5000 ppm), manually phase-corrected and automatically baseline corrected using the Whittaker smoother (Eilers, 2003).

#### 2.1.8. *GC-MS analysis of Papeea capensis extracts and fractions*

All extracts and fractions were subjected to GC-MS analysis to identify different phytoconstituents that can be attributed to the observed antiplasmodial activity. All polar extracts were redissolved in methanol and the lipophilic ones were reconstituted in distilled dichloromethane. Each sample was homogenized by sonication for 30 minutes at 30 °C and prepared to a final concentration of 1µg/ml. All prepared samples were transferred into 1.5 ml short thread vials (La-Pha-Pack, Separations). Gas chromatography and mass spectrometry analysis were conducted on the GCMS-QP2010SE (Shimadzu) machine and an AOC-20i/s autosampler was used to inject the sample into the machine. The start temperature was 30 °C and the end temperature

was 230 °C. The splitless injection technique was conducted for all samples and the injection volume was 1 µl. For the MS analysis, the ion source temperature was 230 °C, the interphase temperature at 250 °C, and the solvent cut time was at 1.5 minutes. The detector voltage was set to 0.1 kV and adjusted to a tuning result. The start time was set to 4.5 minutes and the end time was 30 minutes. The acquisition mode was calibrated to the scan mode, running at a speed of 2500. The mass range was 40 – 650 m/z. Compounds were tentatively identified based on the comparison of their relative mass spectral data with those of the National Institute for Standards and Technology (NIST) database.

### **3. Results and discussions**

*In vitro* antiplasmodial and cytotoxicity, screening was conducted on 30 samples acquired from *P. capensis* twigs. Five extracts (I, II, III, IV, and V) and 25 fractions (A–Y). These were evaluated against the chloroquine sensitive *P. falciparum* NF54 strain and for cytotoxicity screening L-6 rat skeletal myoblast cell lines were used. Selectivity index (SI) values represent the quotient of the antiplasmodial and cytotoxicity IC<sub>50</sub> values (cytotoxicity IC<sub>50</sub> value is divided by antiplasmodial IC<sub>50</sub> value to obtain the SI). A sample was regarded as a potential candidate when its concentration inhibited 50% of the parasite proliferation (IC<sub>50</sub> ≤ 5 µg/ml and SI ≥ 10). A sample's high parasite selectivity index is not tantamount to high antiplasmodial efficacy and the antiplasmodial efficacy of an extract is not a result of its *in vitro* cytotoxicity when SI ≥ 10 (Ndjakou et al., 2007). Table 1 shows the IC<sub>50</sub> values of all assayed extracts and fractions with their respective selectivity index (SI) values.

**Table1.** The antiplasmodial activity (IC<sub>50</sub> values (µg/ml)) and cytotoxicity of *Pappea capensis* extracts and fractions with selectivity index (SI) values computed. Mean values were computed for two independent experiments conducted in duplicate.

Sample ID	Solvent	IC <sub>50</sub> (µg/ml)	Cytotoxicity (µg/ml)	Selectivity Index (SI)
I	DCM	2.93 ± 0.66	40.3 ± 6.36	14
II	dH <sub>2</sub> O	24.25 ± 10.11	>100	ND
III	DCM	2.59 ± 0.51	55.3 ± 3.75	21
IV	MeOH	3.56 ± 0.31	45.1 ± 3.32	13
V	H <sub>2</sub> O	40.40 ± 0.00	65.3 ± 3.11	2
A	DCM	7.90 ± 1.17	>100	ND
B	DCM	2.98 ± 0.31	61.0 ± 8.27	20
C	DCM	1.12 ± 0.02	21.0 ± 2.33	18
D	DCM	0.85 ± 0.03	31.5 ± 16.62	37
E	DCM	0.91 ± 0.00	26.9 ± 11.09	30
F	DCM	2.37 ± 0.08	54.0 ± 1.41	23
G	DCM	3.18 ± 1.27	61.9 ± 6.22	19

H	DCM	$11.27 \pm 2.87$	$15.9 \pm 1.20$	1
I	DCM	$2.81 \pm 0.63$	$51.5 \pm 7.14$	18
J	MeOH	$38.80 \pm 1.82$	>100	ND
K	MeOH	$3.90 \pm 1.15$	$39.0 \pm 13.72$	10
L	MeOH	$4.84 \pm 0.35$	$56.1 \pm 2.69$	12
M	MeOH	$1.56 \pm 0.80$	$56.5 \pm 0.07$	36
N	MeOH	$0.60 \pm 0.48$	$54.3 \pm 2.40$	91
O	MeOH	$21.80 \pm 0.14$	$40.4 \pm 15.13$	2
P	MeOH	$4.09 \pm 1.77$	$50.5 \pm 4.60$	12
Q	MeOH	$24.45 \pm 0.64$	>100	ND
R	dH <sub>2</sub> O	$22.20 \pm 1.13$	$48.2 \pm 5.94$	2
S	dH <sub>2</sub> O	$28.75 \pm 0.78$	$53.0 \pm 0.00$	2
T	dH <sub>2</sub> O	$25.75 \pm 18.03$	>100	ND
U	dH <sub>2</sub> O	$33.95 \pm 21.14$	>100	ND
V	dH <sub>2</sub> O	$20.60 \pm 8.63$	>100	ND
W	dH <sub>2</sub> O	$20.50 \pm 0.00$	>100	ND

X	dH <sub>2</sub> O	20.70 ± 0.00	>100	ND
Y	dH <sub>2</sub> O	22.55 ± 6.72	59.3 ± 0.57	3
Chloroquine		0.0035		
Podophyllotoxin			0.011	

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**Pf-NF54:** *Plasmodium falciparum* NF54 strain

**L6-Cells:** Rat skeletal myoblast cells

**dH<sub>2</sub>O:** Distilled water

**DCM:** Dichloromethane

**MeOH:** Methanol

**ND:** Not detectable

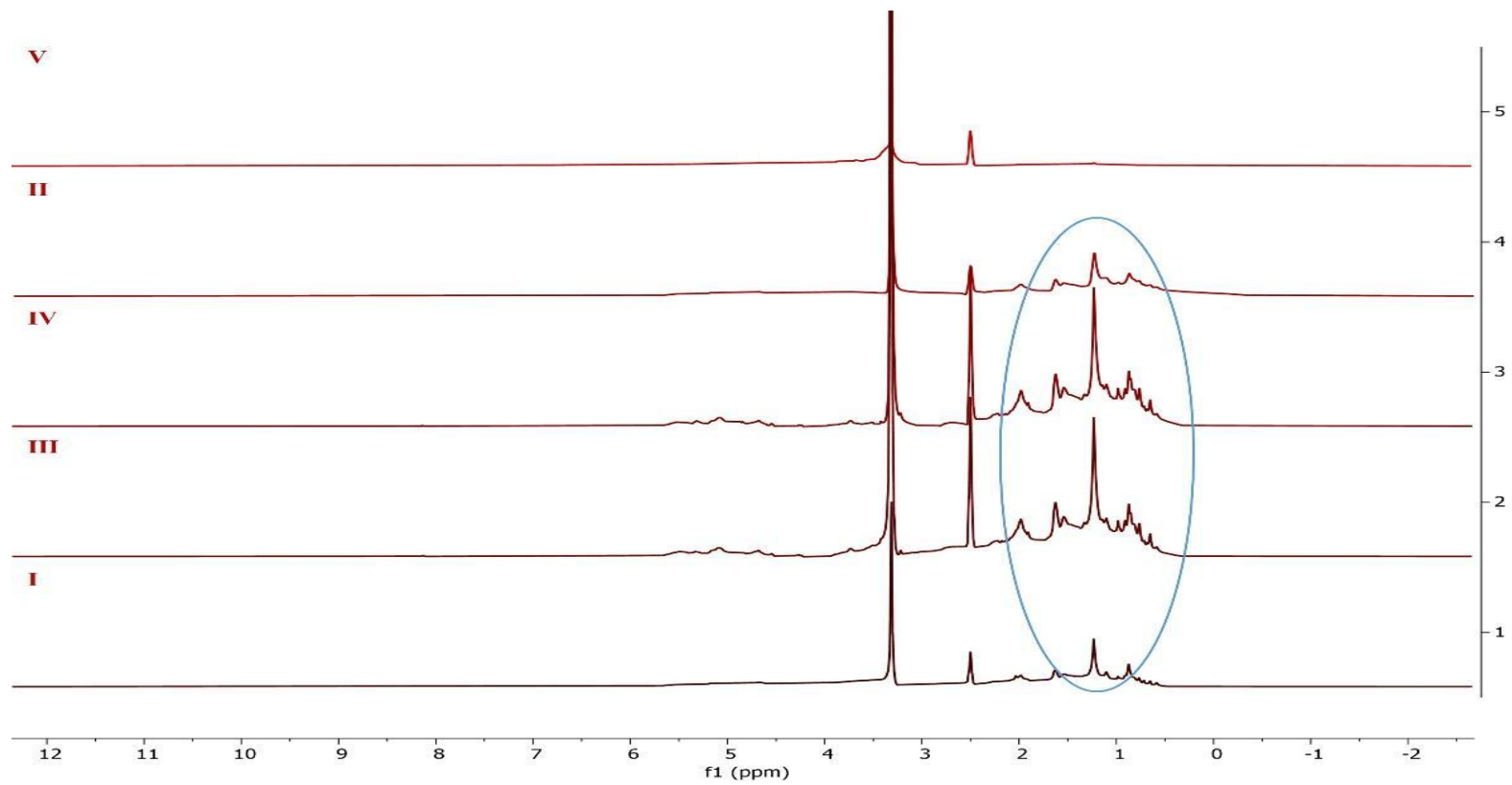
Of the 5 assayed extracts, three (I, III, and IV) exhibited significant antimalarial activity at  $IC_{50} = 2.93, 2.59, \text{ and } 3.56 \mu\text{g/ml}$  and their SI values were 14, 21, and 13, respectively (Table 1). These results are comparable with those reported in previous *in vitro* antiplasmodial activity studies on *P. capensis*. Bapela et al. (2014) extracted from the twigs and reported moderate antiplasmodial activity of the DCM extract at  $IC_{50} = 5.47 \mu\text{g/ml}$  and a SI value of 9.89. Mokoka et al. (2013) extracted from the leaves and roots, reporting significant *Plasmodium* inhibitory effects of the dichloromethane root extract at  $IC_{50} = 5.30 \mu\text{g/ml}$  and an SI value of 16. The noticeable discrepancies in the results can be explained by the different spatial geographic distribution, seasonality, plant part used, and solvent of extraction which plays a critical role in varying the observed antiplasmodial bioactivity in different plant species.

Research conducted in India, on *Aloe* species collected from colder climatic regions recorded high antiplasmodial activity compared to those from warmer regions (Kumar et al., 2017). This is evidence that climatic conditions may have a significant impact of the plants antiplasmodial activity. Different plant parts used to test for antiplasmodial activity also bear different chemical profiles which yield different antiplasmodial results as well. The stem bark assayed by Koch et al., (2005) showed poor inhibitory effects ( $IC_{50} > 10 \mu\text{g/ml}$ ) while the twigs and root extracts from Bapela et al., (2014) and Mokoka et al. (2013) displayed high activity ( $IC_{50} = 5.47 \text{ and } 5.30 \mu\text{g/ml}$  respectively). The methanolic extract IV displayed a significant parasite inhibitory effect at  $IC_{50} = 3.56 \mu\text{g/ml}$  and the SI (Table 1). These results can be compared with the findings of Tajuddeen et al. (2021) who tested the methanolic leaf extract of *P. capensis* and reported poor antiplasmodial activity at lower concentrations ( $10 \mu\text{g/ml}$ ) and more than 80% viability at  $50 \mu\text{g/ml}$  when tested on *P. falciparum* 3D7 strain. The discrepancies in these results can be due to the differing parasite strains used or the

biological assay methods employed. Although there hasn't been extensive research conducted on the antiplasmodial activity of *P. capensis* polar extracts, *in vivo* studies on *Paullinia pinnata* L. which shares the same taxonomic botanical family as *Pappea capensis*, reported poor antiplasmodial inhibitory effects of its methanol leaf extract as well (Adeyemo-Salami et al., 2014). This study is the first to report on the antiplasmodial pharmacological potential of the methanol extract of *P. capensis* twigs. The antiplasmodial activity of the extracts is comparable with results obtained from  $^1\text{H}$  NMR and GC-MS analyses.

The  $^1\text{H}$  NMR spectral data correlates with the observed antiplasmodial biological activity for extracts I, III, and IV. There are some noticeable similarities in their  $^1\text{H}$  NMR spectral region of 0.5 – 2.0 ppm (Figure 1) alluding to a similar class of compounds that can be attributed to observed antiplasmodial bioactivity. Table 2 shows the classes of compounds found in each *P. capensis* extracts. Extracts I, III and IV which displayed the best antiplasmodial activity contain aliphatic, hydrocarbon and alcohol-based class of constituents in abundance. Extracts II and V which have poor antiplasmodial bioactivity contain hydrocarbon and alkyl-based class of constituents in abundance as shown in table 2. Extracts II and V also have the most unknown classes of constituents compared to I, III and IV (Table 2). For purposes of dereplication for unknown compounds with antiplasmodial activity, extract II and IV would best be prioritized. When extracts I – V were stacked and the  $^1\text{H}$  NMR spectra analysed, the most prevalent class of constituents among them was the terpenoids (Figure 1). This was further validated through comparisons made with other studies where terpenoids were analysed using  $^1\text{H}$  NMR and the correlation was established (Kadyrov & Rosiak, 2011; Monakhova et al., 2011).



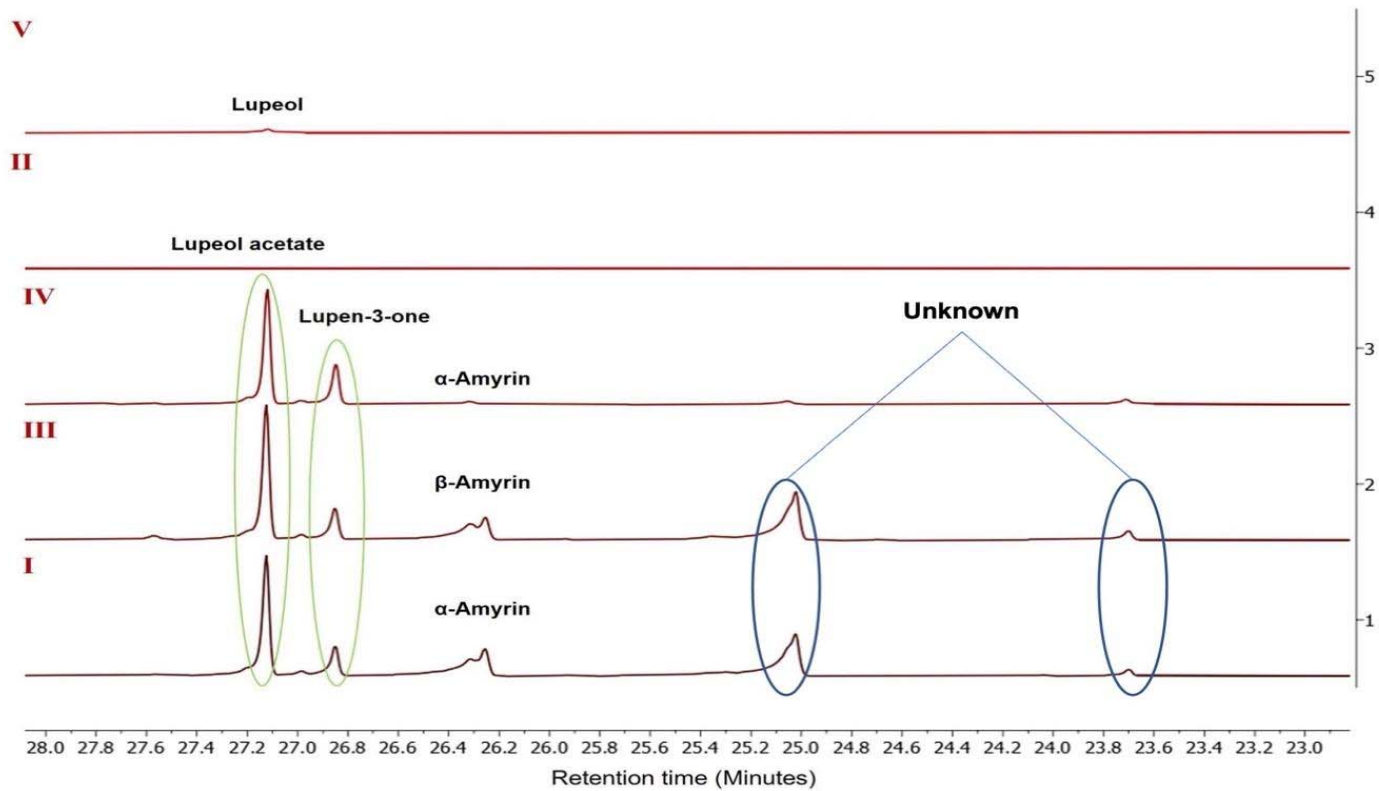


**Figure 1.** Stacked <sup>1</sup>H NMR spectra of *Pappea capensis* extracts showing the different chemical shift similarities highlighted. Extracts I and III are both DCM based; extracts II and V are water based and extract IV is MeOH based.

**Table 2.** The percentage proportion of constituent classes identified in the individual extracts of *Pappea capensis* twigs using GC-MS.

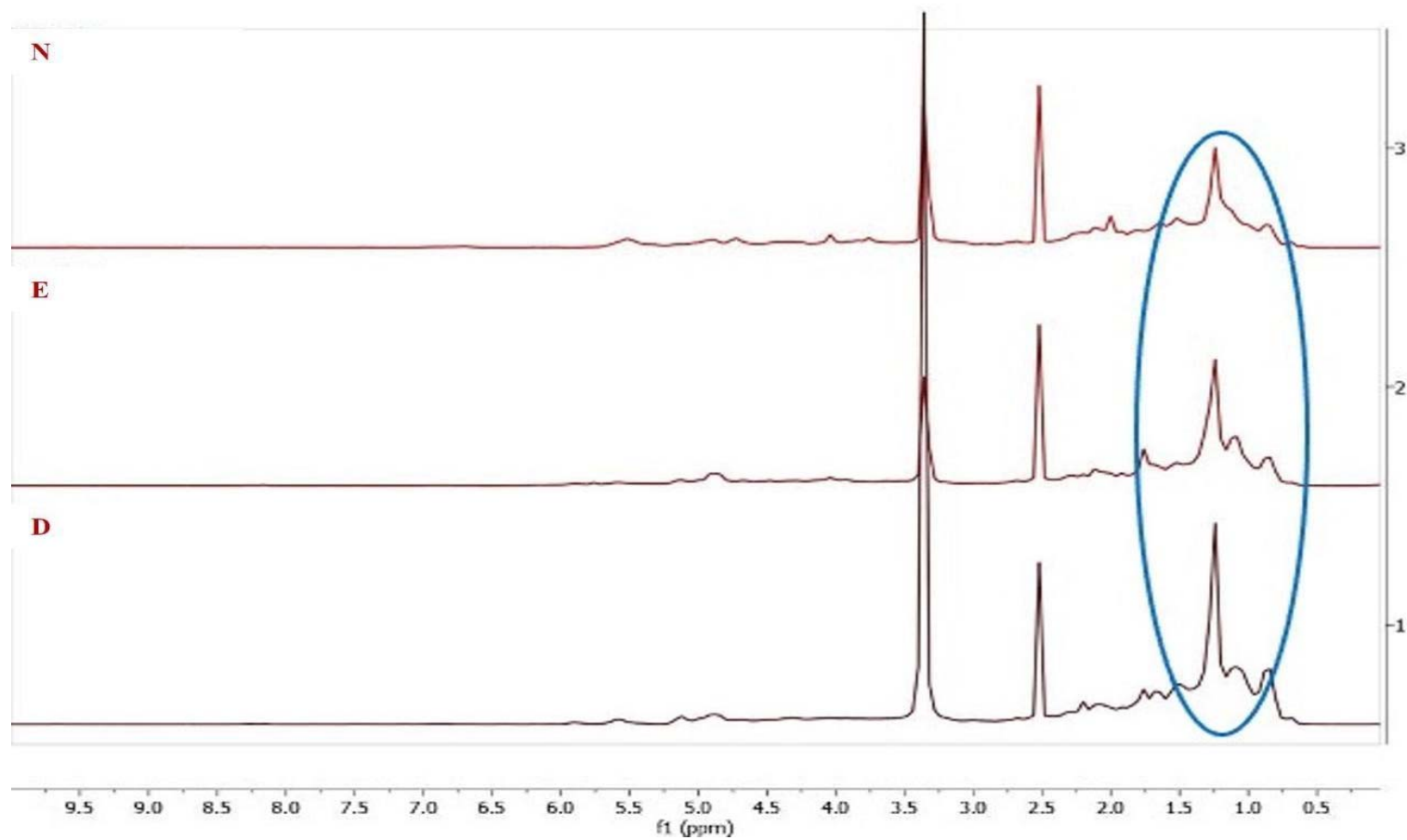
<b>Class</b>	<b>I</b>	<b>II</b>	<b>III</b>	<b>IV</b>	<b>V</b>
Acids	3.15	3.45	2.82	7.51	1.46
Alcohols	10.54	-	8.49	4.06	-
Alkyl	0.85	25.79	0.24	1.51	33.59
Epoxides	-	-	-	0.43	-
Esters	1.3	-	-	2.52	-
Ethers	-	5.2	-	0.43	-
Furan	0.33	-	0.46	0.5	-
Hydrocarbons	36.16	55.07	38.27	16.49	15.43
Sugars	1.52	-	1.24	4.42	38.52
Aliphatic	44.01	-	47.91	62.10	1.53
Unknown	2.14	10.5	0.58	0.03	9.47

From the analysis of the extracts I, III, and IV using GC-MS, the most common constituents among them were identified to be lupen-3-one, lupeol acetate,  $\alpha$ -amyrin, and  $\beta$ -amyrin (Figure 2) (NIST, 2011). Extracts I, III, and IV have different forms of amyirin (extract I contain  $\alpha$ -amyrin and II has  $\beta$ -amyrin), which are abundant in different plant species (Figure 2). Lupeol, lupin-3-one, and lupeol acetate have been reported to possess poor antiplasmodial activity ( $IC_{50} >10 \mu\text{g/ml}$ ), however, they have been reported to have a significant anti-inflammatory activity which is an important malaria-related symptom (Liu et al., 2021; Mwangi et al., 2010). Both  $\alpha$ -amyrin and  $\beta$ -amyrin have established antiplasmodial activities against varying strains of the *Plasmodium* parasite at  $IC_{50}$  values of 0.96 and 2.65  $\mu\text{g/ml}$  respectively (Chung et al., 2009). The presence of these constituents in *P. capensis* extracts can be attributed to its observed antiplasmodial biological activity. Thus, the observed antiplasmodial bioactivity in extracts I, III, and IV correlates with both the GC-MS and  $^1\text{H}$  NMR findings. Extract V reported poor antiplasmodial activity (Table 1), yet indigenous people claim to rely on its decoctions to remedy malaria. An in-depth study into extract V using GC-MS reveals the presence of lupeol, a compound with established antiplasmodial activity (Chung et al., 2009). This compound could exist in low concentrations in extract V hence it is ruled out to possess no activity. This could mean extract V can display antiplasmodial activity at extremely high concentrations. Thus, with the aid of GC-MS, indigenous people using *P. capensis* twigs to treat malaria can be vindicated.

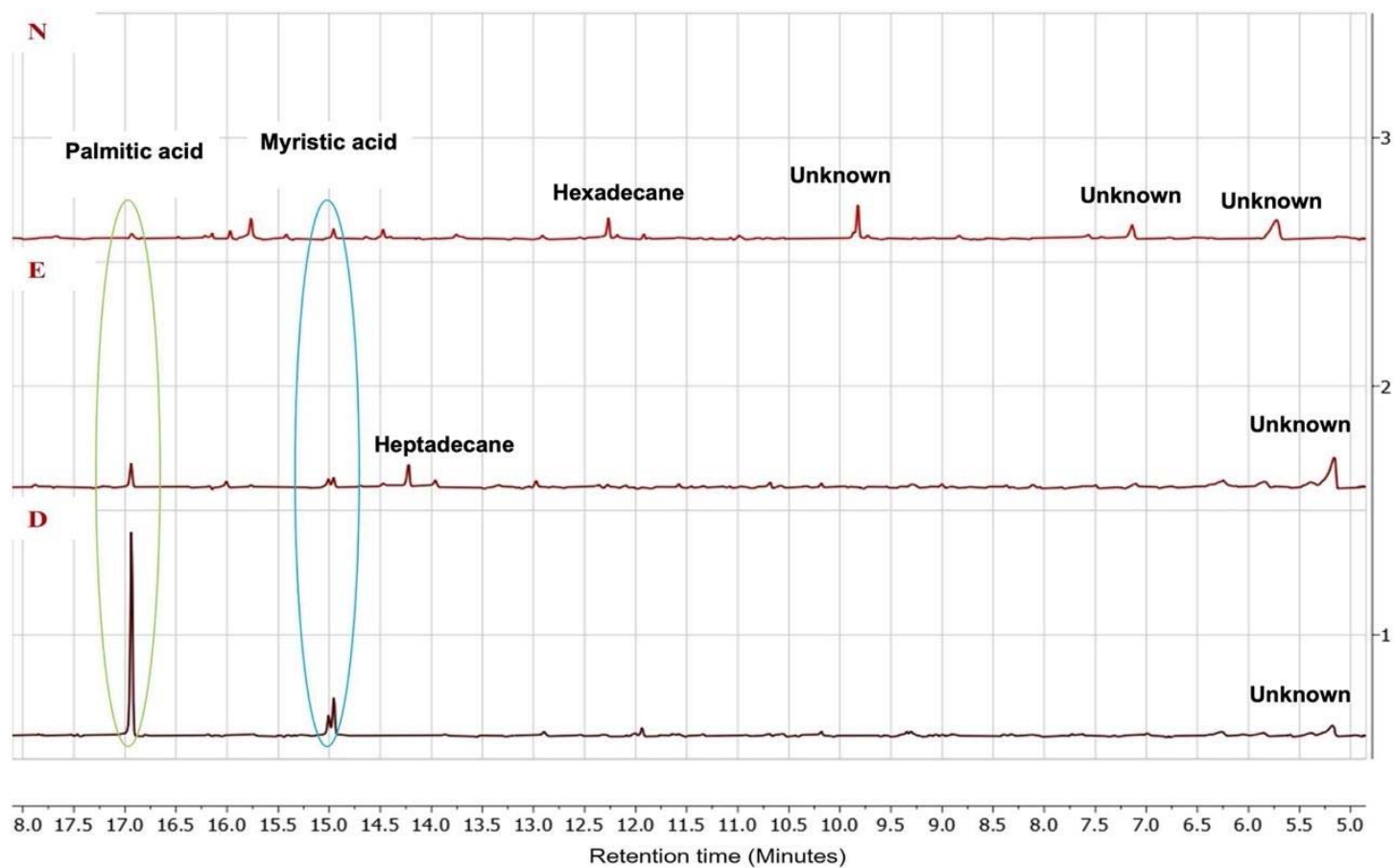


**Figure 2.** The GC-MS spectra of *Papea capensis* extracts. The major phytoconstituents have been highlighted. Extracts I and III are both DCM based; extracts II and V are water based and extract IV is MeOH based.

All fractions recovered from the chromatographic fractionation of extracts II, III, and IV were subjected to antiplasmodial and cytotoxicity screening. Of the 25 tested fractions, only 13 exhibited potential antiplasmodial antiproliferative inhibitory effects against the NF54 strain of *P. falciparum* (Table 1). Fractions N, D and E displayed the highest antiplasmodial bioactivity at IC<sub>50</sub> of 0.60, 0.85 and 0.91 µg/ml respectively (Table 1). Fraction N is methanol based, D and E are both DCM fractions. All the water fraction displayed no antiplasmodial bioactivity (Table 1). The <sup>1</sup>H NMR spectra of fractions N, D, and E were stacked and the regions where the spectra showed similar chemical shifts were integrated and they correlate to the fatty acid-containing phytoconstituents class of compounds (Figure 3). It is common to discover fatty acid containing phytoconstituents that have established antiplasmodial activity and many have been reported to be active against different *Plasmodium* strains (Carballeira, 2008). Gas chromatography-mass spectrometry was also used to underscore similarities and differences among fractions N, D and E to observe similar trends as observed in the <sup>1</sup>H NMR analysis. Fractions N, D, and E shared similar phytoconstituents at 15.0 and 17.0 mins (Figure 4). These peaks were tentatively identified to represent myristic acid and palmitic acid respectively (NIST, 2011). These two fatty acids were previously isolated from *Sonchus arvensis* L., a plant with established antiplasmodial activity (Dwi et al., 2020; Wahyuni et al., 2021). These constituents have not been tested for antiplasmodial activity, however, their occurrence in a plant with established antimalarial activity suggests they can potentially possess the desired antiplasmodial pharmacological activity. Further antiplasmodial bioactivity tests of myristic and palmitic fatty acids should be conducted to ascertain their antiplasmodial activity and cytotoxicity.



**Figure 3.** <sup>1</sup>H NMR spectra of the most active fractions of *Pappea capensis*. Fractions D and E are DCM based and fraction N is MeOH based.



**Figure 4.** Stacked GC-MS spectra of the most active fractions of *Papea capensis* showing myristic acid and palmitic acid. Fractions D and E are DCM based and fraction N is MeOH based.

Phytochemical studies conducted on *P. capensis* reported the occurrence of many constituent classes like terpenes, phenolics, tannins, flavonoids, and alkaloids (Karau et al., 2012). Constituent classes like alkaloids, terpenoids, flavonoids and saponins have been reported in previous studies to have antiplasmodial activity (Akanbi, 2015; Bekono et al., 2020; Ganesh et al., 2012; Uzor, 2020). The observed antiplasmodial activity in *P. capensis* can be further attributed to terpenoid and fatty acid-containing phytoconstituents class of compounds. The results also suggest the possibility that the observed antiplasmodial activity may be attributed to a single or many pure compounds. This can be explained and extrapolated from the observed improvement in *Plasmodium* inhibitory effects when *P. capensis* extracts were purified to less complex fractions. When extract I was reduced to II by liquid-liquid separation, an increase in antiplasmodial activity from 2.93 to 2.59 µg/ml was observed (Table 1). A similar trend was observed when extract III and IV were further purified through column chromatographic fractionation, resulting in N, D and E which also displayed significant improvement in antiplasmodial activity. This may provisionally explain the fact that individual phytoconstituents could be major antiplasmodial activity contributors in *P. capensis*. The results obtained in the study will be instrumental in informing rural people who rely on the use on natural remedies to treat malaria about the antiplasmodial potential of the different extracts and chromatographic fractions. This will assist them in using the plants efficiently and to derive the best pharmacological responses from their use. The detected unknown compounds will be further explored in attempt to isolate new antiplasmodial compounds that have not been discovered, which may contribute to the current list of antimalarial drug hits.



#### 4. Conclusion

Malaria is manageable and curable. Despite the huddles faced by most countries in the sub-Saharan region when it comes to eradicating malaria, this study contributes chemical scaffolds that can be incorporated in the delivery of effective chemotherapeutic agents. It validates the ethnomedicinal use of *P. capensis* in treating malaria and its potential to yield invaluable antiplasmodial hits. Unknown constituents in active extracts will need to be prioritized for isolation and antiplasmodial pharmacological viability tests to dereplicate known compounds with established antiplasmodial activity. Further chemical analysis and phytoconstituent isolation are underway, in attempt to discover unknown antiplasmodial phytochemicals.

#### 5. Credit authorship statement

**Mcebisi Junior Mabuza:** Conceptualized and drafted the entire manuscript. Analysed and interpreted all data. **Marcel Kaiser:** Conducted the antiplasmodial and cytotoxicity biological assays. **Mahwahwatse Johanna Bapela:** Reviewed and edited the manuscript. All authors read and approved the final version of the manuscript.

#### 6. Declaration of competing interests

Authors declare no conflict of interests.

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