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Original Research

Computational, chemical profile and *in vitro* acaricidal property of methanol and chloroform extracts of *Cymbopogon citratus* Stapf leaf

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ABSTRACT

This study investigated the phytochemical composition, antioxidant and acaricidal properties of methanol and chloroform extracts of *Cymbopogon citratus* leaf. A known quantity of the pulverized plant material was first exhaustively extracted with chloroform and thereafter, with methanol using a Soxhlet apparatus set at 40 °C. The extracts were concentrated in a hot air oven at 40 °C. Gas chromatography-mass spectroscopy was used to determine the phytochemical composition, while 1,1 diphenyl-2-picrylhydraxyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assay were used to evaluate the antioxidant profile of the extracts at concentrations of 25 - 400 µg/mL. Contact method was used to investigate the acaricidal property and molecular docking analysis of selected identified compounds were performed against acetylcholinesterase to check their drug like potentials. Fifty-seven and sixty-four compounds were identified in the methanol and chloroform extracts, respectively. The methanol extract produced higher antioxidant and acaricidal properties ($P < 0.05$) relative to the chloroform extract. The compounds formed hydrogen bonds with amino acid residues. In conclusion; the methanol extract demonstrated better antioxidant and acaricidal properties than the chloroform extract, which could be linked to the higher concentration of essential oil (Carvomenthol, (-)-carvone, and eucalyptol) in the methanol extract.

Keywords: Acaricidal activity, antioxidant, *Cymbopogon citratus*, molecular docking, phytochemical composition

INTRODUCTION

Tick is an external parasite of public health and veterinary importance especially in the tropics. It negatively impacts the production and health of animals either directly (via their bite) or indirectly (through the transmission of disease causing agents like viruses, protozoa, bacteria and rickettsiae) (Rego *et al*., 2019). The tick bite and sucking of blood in the infested animals cause anaemia, irritation, and inflammation (dermatitis) (Brites-Neto *et al*., 2015; Shiferaw, 2018). The anaemia and discomfort associated with the activities of the ticks on the host may lead to oxidative stress and immune suppression which can predispose to secondary bacterial infection and myiasis (Heyman *et al*., 2010; Man *et al*., 2022). The attendant impact of these effects is losses in productivity like reduced meat and milk yield, morbidity and mortality in extreme cases (Hurtado *et al*., 2018). The global economic losses attributed to ticks and tick-borne diseases is estimated at US$22–30 billion/annum (Lew-Tabor and Valle, 2016; Singh *et al*., 2022).

The control of ticks in the farm animal and the environment has been via the use of synthetic acaricides, substances or mixtures that prevent, destroy, repel or mitigate pests (Adenubi *et al*., 2018). The most commonly used synthetic compounds are organophosphates, synthetic pyrethroids, amitraz and ivermectin (Valsoni *et al*., 2020). A number of

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problems associated with the use of these products, such as high cost, environmental pollution, rapid resistance of the species and damaging of their natural predators have limited the use of these acaricides (Adenubi *et al*., 2018). An alternative to the synthetic acaricides is the use of plant oils and extracts and the relevant derived products, due to the compatibility with the natural predators, having short residual effects and low toxicity to man (Camilo *et al*., 2018). One of the plants traditionally used to control insect pest is *Cymbopogon citratus*.

Cymbopogon citratus, commonly called "lemon grass", "citronella grass", "aromatic grass" and "squinant", belongs to the family Poaceae. It is a perennial grass with many stem arising from a short rhizomatous rootstock (Shah *et al*., 2011). It is found in semi-temperate and tropical regions of Asia, America and Africa (Ranade and Thiagarajan, 2015). It is rich in essential (citral oil and others), which imparts its fragrance. The plant grows up to 1.8 m high and 1.2 m width, with narrow leaves that measures 1.3-2.5 cm width and 1 m in length (Haque *et al*., 2018). It is significantly used in cosmetics and food industries as well as ethnomedicine. The plant has been reported to possess some phytochemicals like flavonoids, phenolic compounds, terpenes, alkaloids and glycosides (Haque *et al*., 2018). The decoction and tea prepared from the leaves of *C. citratus* are used as antiplasmodic, antipyretic, anti-inflammatory, analgesic, sedative and diuretic (Haque *et al*., 2018). The fresh leaves are used as snake, insect, and tick repellent in traditional medicine, but there is a dearth of information on its acaricidal properties. This study was designed to investigate the phytochemical composition, antioxidant and acaricidal properties of methanol and chloroform extracts of *Cymbopogon citratus* leaf.

MATERIALS AND METHODS

PLANT COLLECTION AND IDENTIFICATION

Fresh leaves of *Cymbopogon citratus* (Lemon grass) was harvested/collected in May, 2021 from Ndoru in Ikwuano Local Government Area of Abia State. The plant sample was identified by Prof. M. C. Dike of College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Nigeria. A voucher sample (MOUAU/VPP/2021/04) was kept at the Department of Veterinary Physiology and Pharmacology herbarium.

EXTRACT PREPARATION

The fresh leaves were sorted, washed with tap water and air dried on the laboratory bench. They were turned daily to prevent mold growth. The dry samples were later pulverized into coarse powder. A weighed quantity of the pulverized plant material was first exhaustively extracted with chloroform and thereafter, with methanol using a Soxhlet apparatus set at 40 °C. The extracts were concentrated in a hot air oven at 40 °C.

ANTIOXIDANT STUDY 1,1 DIPHENYL-2-PICRYLHYDRAXYL (DPPH) RADICAL SCAVENGING ASSAY

The free radical scavenging capacity of the chloroform and methanol extracts of *C. citratus* leaf were evaluated as described in a standard protocol (Egua *et al*., 2020). Two (2) ml of the extracts at varied concentrations (25 to 400 µg/mL) in triplicate were mixed with one (1) ml of 0.5mM DPPH (in methanol) in a cuvette. The absorbance (Abs) at 517 nm was measured after 30 min of incubation in the dark at room temperature. The percentage antioxidant activity was calculated as follows:

% antioxidant activity (AA) =

$$\frac{Abs\ control - (Abs\ sample - Abs\ blank)}{Abs\ control} \times \frac{100}{1}$$

A mixture of 1.0 ml of methanol plus 2.0 ml of the extract was used as the blank while 1.0 ml of the 0.5mM

DPPH solution plus 2.0 ml of methanol was used as the negative control while ascorbic acid was used as a standard reference (Egua *et al*., 2020).

FERRIC REDUCING ANTIOXIDANT POWER (FRAP) ASSAY

Ferric reducing ability of the chloroform and methanol extracts of *C. citratus* leaf, which measures the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe (II)-TPTZ) at acid medium, was carried out as described by Benzie and Strain (1999). Briefly, acetate buffer 300 mmol/pH 3.6 (3.1 g sodium acetate trihydrate and 16 ml concentrated acetic acid per 1000 ml) was prepared. Then 10 mmol/L of 2,4,6-tripyridyl-8-triazine (TPTZ) in 40 mmol/L HCl was constituted and thereafter, 20 mmol/L of $FeCl_3$.6H2O in distilled water was also constituted.

FRAP working solution: 120 ml acetate buffer, 12 ml TPTZ solution and 12 ml FeCl3 .6H2O solutions were mixed.

The FRAP reagent (3 mL) and 100 µl sample solution at concentrations of 25, 50, 100, 200 and 400 µg/mL were mixed and allowed to stand for 4 minutes. The absorbance was recorded at 593 nm, at 37°C. The ascorbic acid was tested in a parallel process. The absorbance of each test tube was taken at 0 and 4 minutes after addition of sample.

FRAP value = $abs\ 4\ \text{minutes} - abs\ 0\ minute$

TICK COLLECTION

Ethical approval was obtained from MOUAU research ethical committee (No: MOUAU/CVM/REC/202314). Adult *Amblyomma variegatum* ticks were collected from the cattle ranch of Michael Okpara University of Agriculture Umudike between early June 2021 to late July 2021. Ticks collected included adult *A. variegatum* male and female ticks

encountered. All adult male ticks encountered were collected but only females that had not significantly fed (≤ 6 mm in size) were collected. Collected ticks were placed in perforated plastic containers with fresh leaf to maintain moisture. Species identification and sex differentiation of ticks were done in the Department of Veterinary Parasitology and Entomology Laboratory using the pictorial guide provided by Walker *et al.* (2007). Engorged females were not collected for this study as their larger size compared to males may produce data which might be inconsistent with the probit model employed in the analysis (Robertson *et al*., 2007; Muyobela *et al*., 2016). The collected ticks were used within 48 hours.

ACARICIDAL ASSAY (FREE CONTACT METHOD)

The acaricidal properties of the extracts were tested using the free contact method (Muyobela *et al*., 2016). Fifteen (15) adult ticks were placed in each treatment and control groups in petri dish. The experiment was conducted in triplicates. A paste of the extract was placed on a filter that was used to cover the surface of petri dish (through capillarity, the extract spread out to the entire filter paper) while the ticks are allowed to roam freely to have contact with the extract. Ticks were then prevented from crawling out of the petri dish by using mesh and rubber bands. Tick mortality was then recorded after 0.5, 1, 2, 3, and 24 hours post introduction. Diazinon was used as reference standard.

Mortality in bioassays was calculated using the formula Mortality (%) = $\frac{Dead\ tick\ count}{Total\ tick\ count} \times 100$ for each replicate. In free contact bioassay, mean mortality for control and treatment levels was pooled using the formula

PMM = $\frac{mortality\ i+mortality\ ii+mortality\ iii}{3}$ where i, ii, and iii denote the 1st, 2nd and 3rd sample data, respectively.

GAS CHROMATOGRAPHY-MASS SPECTRA (GC-MS) ANALYSIS

Agilent Technologies GC systems with GC-7890A/MS-5975C model (Agilent Technologies, Santa Clara, CA, USA) equipped with HP-5MS column (30 m in length 250 m in diameter 0.25 m in film thickness) were used for GC-MS analysis of bioactive compounds from different extracts. GC-MS spectroscopic identification required an electron ionization system that used electrons with a high energy (70 eV). The gas that served as the carrier was pure helium gas (99.995%) at a flow rate of 1 mL/min. The start temperature was set at 50-150 °C, with a 3 °C/min increase rate and a holding period of about 10 minutes. Finally, the setting was raised to 300 °C at a rate of 10 °C/min. In a splitless mode, one microliter of the prepared 1% extracts diluted with respective solvents was injected. The relative amount of chemical compounds present in each extract was expressed as a percentage based on the peak area produced in the chromatogram. Based on GC retention time on the HP-5MS column and spectral matching with computer software data of standards (Replib and Mainlab data of GC-MS systems), bioactive compounds extracted from various extracts were identified.

MOLECULAR DOCKING

The structures of the six major compounds in the plant extract carvomenthol, (-)-carvone, hemellitol, eucalyptol, decalin, cyclohexadecane were downloaded from the PubChem database (PubChem (nih.gov)), and docked against acetylcholinesterase downloaded from the protein data bank (PDB) (RCSB PDB: Homepage). Docking was achieved using Autodock Vina (Trott and Olson, 2010; Emori *et al*., 2022; Ejiofor *et al*., 2023) and Biovia Discovery Studio to investigate the application of these compounds as potential acaricidal agents. Briefly, the three-dimensional structure of acetylcholinesterase (PDB ID: 4PQE) was retrieved from protein data bank as target. The chemical structure of sarin used as standard acetylcholinesterase inhibitor was obtained from the PubChem database. Finally, BIOVIA-DSV software was used to visualize the active amino acid residues of individual protein-ligand docking complexes (Oyedemi *et al.,* 2021; Agwamba *et al*., 2022).

STATISTICAL ANALYSIS

Data were expressed as mean ± standard error of the mean (mean ± SEM). Statistical analysis was performed by one way-analysis of variance (one way ANOVA) at 95 % confidence level using SPSS statistical software. Mean differences were separated using Least Significant Different (LSD).

RESULTS

ACARICIDAL PROPERTY

The result of the acaricidal effects of the methanol and chloroform extracts of *C. citratus* leaf against *Amblyomma variegatum* is presented in Table I. The CCME produced time-dependent increase in percentage mortality of the tick which was significantly ($P < 0.05$) when compared with the control. At 24 h post treatment, the control, CCME, CCCE and diazinon produced 0.00%, 86.67%, 6.67% and 100% mortality in the treated tick population.

THE GC-MS ANALYSIS OF THE EXTRACTS

The result of the GC-MS analysis of the CCME and CCCE are presented in Tables II and III respectively. The GC-MS detected a total of 57 compounds in the CCME and only six of the identified compounds had above 2% composition (Table II). These compounds are: 3-Ethyltoluene (2.37%), Hemellitol (2.58%), (-)-Carvone (9.36%), 1-Nonadecene (3.44%), Oleic Acid (22.08%) and 2-Methyl-Z,Z-3,13-octadecadienol (17.77%).

The GC-MS detected a total of 64 compounds in the CCCE and only 15 of the identified compounds had above 2% composition (Table III). These compounds include: butyl 9-octadecenoate (2.06%); 1-hexadecanesulfonic acid, 3,5-dichloro-2,6-dimethyl-4-pyridyl ester (2.33%); 3-methylhexadecane (3.38%); 2-methyltricosane (2.30%); 3,8-dimethyldecane (9.12%); undecane (3.19%); dodecane (4.85%); 2,6,10,14-tetramethylheptadecane (4.71%); carbonic acid, nonyl vinyl ester (4.85%); 2,6-dimethylheptadecane (4.51%); tridecane (3.15%); 2,4-di-tert-butylphenol (8.08%); Z-8-hexadecene (2.35%); 1-octadecene (5.06%) and oleic acid (3.72%).

Table 1: Acaricidal effects of the methanol and chloroform extracts of *C. citratus* leaf against *Amblyomma variegatum* . Percentage mortality of the ticks (± SEM)

Samples	0.5 h	1 h	2 h	3 h	24 h
CONTROL	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
CCME	0.00 ± 0.00	0.00 ± 0.00	46.67 ± 6.67*	46.67 ± 6.67*	86.67 ± 13.33*
CCCE	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	6.67 ± 6.67*
Diazinon	86.67 ± 13.33*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*	100.00 ± 0.00*

*P < 0.05 when compared with the control, CCME = *C. citratus* methanol extract, CCCE = *C. citratus* chloroform extract

ANTIOXIDANT ASSAY

The results of the *in vitro* antioxidant potential of the CCME and CCCE using DPPH radical scavenging and FRAP assay are presented in Figure I and II, respectively. The extracts produced concentration dependent increase in DPPH radical scavenging activity and the CCME produced significantly (P < 0.05) higher antioxidant effect when compared with CCCE (Figure I). CCME had antioxidant values of 29.80, 47.42, 83.16, 92.53 and 92.35 compared CCCE with 6.08, 7.77, 13.11, 21.03 and 35.56 at the same concentrations of 25, 50, 100, 200 and 400 µg/ml. At 200 and 400 µg/ml, the DPPH radical scavenging effects of CCME were comparable to the ascorbic acid.

The extracts produced concentration dependent increase in FRAP value and the CCME produced significantly (P < 0.05) higher FRAP value when compared with CCCE, but lower (P < 0.05) when compared with ascorbic acid (Figure II). CCME was found to have 0.14 and 0.32 FRAP values relative to 1.59 and 1.96 FRAP values with ascorbic acid at 200 and 400 µg/ml.

MOLECULAR DOCKING

The binding affinity (docking score) measured in kcal/mol of the compounds and standard drugs (diazinon) against a potential protein target for ticks and mites is presented in Table IV. Result showed that carvomenthol, (-)-carvone, hemellitol, eucalyptol, decalin, and cyclohexadecane exhibited a docking score of {4PQE: -6.50, -6.60, -6.60, -6.30, -6.50, -8.60 respectively vs -7.50 for diazinon} towards the investigated target protein compared to standard drug. Among the studied compounds, cyclohexadecane showed the highest docking score (-8.60 Kcal/mol). The compounds formed conventional hydrogen bonds with amino acid residues. Carvomenthol formed conventional hydrogen bond with SER293, (-)-carvone formed hydrogen bonding with PHE295, while eucalyptol formed hydrogen bond with TRY337 and the standard agent (diazinon) formed hydrogen bond with THR83 and TYR124 (Figure III).

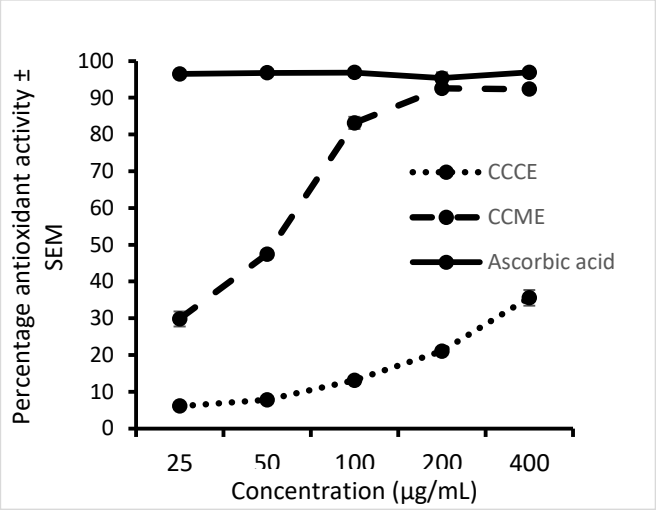

Figure I: The DPPH radical scavenging effects of CCCE and CCME

Legend: CCME = *C. citratus* methanol extract, CCCE = *C. citratus* chloroform extract

Table II: The compounds detected by GC-MS in the CCME

S/N	% conc	RT	Chemical name	Molecular Formular	Molecular mass
1	0.33	5.219	9-Eicosene, (E)-	$C_{20}H_{40}$	280.50
2	1.34	5.88	3,7,11,15-Tetramethylhexadecan-1-ol	$C_{20}H_{42}O$	298.50
3	2.37	5.485	3-Ethyltoluene	C_9H_{12}	120.19
4	1.28	5.627	4,5-dimethyl-, [R*,S*-(Z)]-2-undecene	$C_{13}H_{26}$	182.00
5	2.58	5.693	Hemellitol	C_9H_{12}	120.19
6	0.92	5.919	trans-1,3-Dimethylcyclohexane	C_8H_{16}	112.21
7	0.63	6.372	Decane	$C_{10}H_{22}$	142.29
8	0.21	6.972	3-tridecyl chloroacetate	$C_{15}H_{29}ClO_2$	276.84
9	0.60	7.087	1-Octadecene	$C_{18}H_{36}$	252.50
10	1.80	7.204	Eucalyptol	$C_{10}H_{18}O$	154.25
11	0.27	7.287	3,7,11,15-tetramethyl-hexadecyl acetate	$C_{22}H_{44}O_2$	340.60
12	1.32	7.416	1-Fluorononane	$C_9H_{19}F$	146.25
13	0.59	7.585	4-Trifluoroacetoxyhexadecane	$C_{18}H_{33}F_3O_2$	338.40
14	1.82	7.817	Decalin	$C_{10}H_{18}$	138.25
15	1.33	7.949	1-Docosene	$C_{22}H_{44}$	308.60
16	0.60	8.042	17-Pentatriacontene	$C_{35}H_{70}$	490.90
17	0.51	8.101	1-ethyl-3,5-dimethyl-benzene,	$C_{10}H_{14}$	134.22
18	1.35	8.135	cyclobutyl heptadecyl oxalate	$C_{23}H_{42}O_4$	382.60
19	1.02	8.320	E-14-Hexadecenal	$C_{16}H_{30}O$	238.41
20	0.56	8.675	2,4-Dimethyl-1,5-diazabicyclo[3.1.0]hexane (cis)	$C_6H_{12}N_2$	112.17
21	0.42	8.777	4-Trifluoroacetoxytetradecane	$C_{16}H_{29}F_3O_2$	310.39
22	0.84	8.834	2,2-Dimethyl-4,6-dioxo-5-(O-phenyl endiamin-N-methylidene)-1,3-dioxan		
23	0.54	9.176	Undecane	$C_{11}H_{24}$	156.31
24	0.32	9.415	trans-Decalin, 2-methyl-	$C_{11}H_{20}$	152.28
25	0.14	9.877	1-ethyl-2,3-dimethyl-benzene	$C_{10}H_{14}$	134.22
26	0.12	10.128	1-methyl-3-(1-methylethyl)-cyclopentane	C_9H_{18}	126.24
27	0.16	10.695	2-ethenyl-1,4-dimethyl-benzene	$C_{10}H_{12}$	132.20
28	0.26	10.809	undecyl Pentadecafluorooctanoate	$C_{14}H_{23}F_5O_2$	318.32
29	0.16	10.909	Nonyl tetradecyl ether	$C_{23}H_{48}O$	340.60
30	0.16	11.032	5-Ethyl-2-methyloctane	$C_{11}H_{24}$	156.31
31	0.72	11.611	Carvomenthol	$C_{10}H_{20}O$	156.27
32	0.69	11.797	Cyclotridecane	$C_{13}H_{26}$	182.35
33	1.81	12.096	Dodecane	$C_{12}H_{26}$	170.33
34	0.87	13.336	5-methyl-2-(1-methylethylidene)-cyclohexanone	$C_{10}H_{16}O$	152.23
0	9.36	13.507	(-)-Carvone	$C_{10}H_{14}O$	150.22
36	0.17	14.840	Carane	$C_{10}H_{18}$	138.25
37	0.14	17.573	Cyclotetradecane	$C_{14}H_{28}$	196.37
38	0.58	17.688	Tetradecane	$C_{14}H_{30}$	198.39
39	0.46	21.166	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32
40	1.62	22.753	Hexadecane	$C_{16}H_{34}$	226.41
41	0.19	25.097	7-Propyltridecane	$C_{16}H_{34}$	226.44
42	0.32	27.323	Octadecane	$C_{18}H_{38}$	254.49
43	0.09	29.538	Methyl-palmitate	$C_{17}H_{34}O_2$	270.45
44	0.28	30.224	5-Eicosene, (E)-	$C_{20}H_{40}$	280.50
45	1.93	31.072	Triacontyl acetate	$C_{32}H_{64}O_2$	480.85
46	0.49	31.110	Cyclohexadecane	$C_{16}H_{32}$	224.43

S/N	% conc	RT	Chemical name	Molecular Formular	Molecular mass
47	0.98	31.155	9-Octadecenoic acid, (E)-	$C_{18}H_{34}O_2$	282.46
48	0.25	31.287	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.50
49	0.58	31.340	Methyl octadec-11-enoate	$C_{19}H_{36}O_2$	296.50
50	0.41	31.527	1-Eicosene	$C_{20}H_{40}$	280.50
51	3.44	31.767	1-Nonadecene	$C_{19}H_{38}$	266.50
52	0.66	32.017	1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-Cyclohexane	$C_{20}H_{40}$	280.53
53	0.16	32.914	9-Hexacosene	$C_{26}H_{52}$	364.69
54	0.16	33.519	But-2-yn-1-yl eicosyl carbonate	$C_{25}H_{46}O_3$	394.60
55	1.75	33.950	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.56
56	22.08	34.762	Oleic Acid	$C_{18}H_{34}O_2$	282.47
57	17.77	36.089	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.50

Legend: % conc = Percentage composition; RT = Retention time; S/N = Serial number; CCME = *Cymbopogon citratus* methanol extract

Table III: The compounds detected by GC-MS in the CCCE

S/N	% conc	RT	Chemical name	Molecular Formular	Molecular mass
1	2.06	5.250	Butyl 9-octadecenoate	$C_{22}H_{42}O_2$	338.60
2	0.19	5.455	3-tridecyl-2-thiopheneacetate	$C_{19}H_{32}O_2S$	324.50
3	1.64	6.885	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.50
4	0.10	7.137	4-nitrophenyl palmitate	$C_{22}H_{35}NO_4$	377.52
5	0.10	7.225	3-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.50
6	1.33	7.425	1,3-dichloro-benzene	$C_6H_4Cl_2$	147.00
7	0.53	7.788	1-(ethenyloxy)-octadecane	$C_{20}H_{40}O$	296.53
8	1.41	8.521	2-Ethyl-1-dodecanol	$C_{14}H_{30}O$	214.39
9	2.33	8.612	3,5-dichloro-2,6-dimethyl-4-pyridyl-1-hexadecanesulfonate	$C_{23}H_{39}Cl_2NO_3S$	480.53
10	3.38	8.876	3-Methylhexadecane	$C_{17}H_{36}$	240.50
11	1.84	8.980	Nonane	C_9H_{20}	128.20
12	1.19	9.059	Decane	$C_{10}H_{22}$	142.29
13	1.69	9.138	2,6-Dimethyldecane	$\mathbf{C_{12}H_{26}}$	170.33
14	2.30	9.217	2-methyltricosane	$C_{24}H_{50}$	338.70
15	9.12	9.430	3,8-Dimethyldecane	$\mathbf{C_{12}H_{26}}$	170.33
16	3.19	9.622	Undecane	$C_{11}H_{24}$	156.31
17	4.85	9.737	Dodecane	$\mathbf{C_{12}H_{26}}$	170.33
18	4.71	9.816	2,6,10,14-tetramethylheptadecane	$C_{21}H_{44}$	296.60
19	1.43	9.938	Nonyl prop-1-en-2-yl carbonate	$C_{13}H_{24}O_3$	228.33
20	4.85	10.159	Nonyl vinyl carbonate	$C_{12}H_{22}O_3$	214.30
21	4.51	10.268	2,6-Dimethylheptadecane	$C_{19}H_{40}$	268.50
22	1.80	10.506	5-methylundecane	$\mathbf{C_{12}H_{26}}$	170.33
23	1.59	10.635	3-ethyloctane	$C_{10}H_{22}$	142.28
24	0.66	10.992	Stearic acid hydrazide	$C_{18}H_{38}N_2O$	298.50
25	0.15	11.468	Tetradecyloxirane	$C_{16}H_{32}O$	240.42
26	0.28	11.563	1-Octadecanesulphonyl chloride	$C_{18}H_{37}ClO_2S$	353.00
27	0.95	12.584	E-14-Hexadecenal	$C_{16}H_{30}O$	238.41
28	0.24	13.191	Trifluoroacetoxy hexadecane	$C_{18}H_{33}F_3O_2$	338.40
29	0.59	14.888	Decyl octyl ether	$\mathbf{C_{18}H_{38}O}$	270.50
30	3.15	15.706	Tridecane	$C_{13}H_{28}$	184.36
31	0.84	15.873	1,4-Dihydro-1,4-methanonaphthalene	$C_{11}H_{10}$	142.20
32	0.53	16.361	9-Oxabicyclo[6.1.0]nonane	$C_8H_{14}O$	126.20
33	0.26	17.473	1-chlorohexadecane	$\mathbf{C_{16}H_{33}Cl}$	260.90
34	0.45	17.662	13-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.50
35	0.23	17.804	Decyloxirane	$C_{12}H_{24}O$	184.32
36	1.31	18.283	4-Tetradecene, (Z)-	$C_{14}H_{28}$	196.37
38	0.67	18.963	2,7-dimethyl-naphthalene	$C_{12}H_{12}$	156.22
39	0.23	19.354	Citronellol	$C_{10}H_{20}O$	156.27
40	0.15	19.743	1-Hexacosanol	$C_{26}H_{54}O$	382.71
41	0.46	20.087	9-Octadecenal	$C_{18}H_{34}O$	266.50
42	0.16	20.899	1,1'-oxybis-dodecane,	$C_{24}H_{50}O$	354.65
43	0.88	21.104	2-methylundecane	$C_{12}H_{26}$	170.33
44	8.08	22.130	2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	206.32
45	2.35	23.440	Z-8-Hexadecene	$\mathbf{C_{16}H_{32}}$	224.42

S/N	% Conc.	RT	Chemical name	Molecular Formular	Molecular mass
46	0.57	23.597	Hexadecane	$C_{16}H_{34}$	226.41
47	0.31	23.743	Cetene	$C_{16}H_{32}$	224.43
48	0.30	23.978	Cyclohexadecane	$C_{16}H_{32}$	224.43
49	0.32	25.513	Methyl-4-(3-hydroxy-3-methylbut-1-ynyl)benzoate	$C_{13}H_{14}O_3$	218.25
50	0.26	25.958	2-hydroxy-cyclopentadecanone	$C_{15}H_{28}O_2$	240.38
51	0.41	26.072	2,6-Dimethylheptane	C_9H_{20}	128.25
52	5.06	28.090	1-Octadecene	$C_{18}H_{36}$	252.50
53	0.15	28.553	3-Eicosene, (E)-	$C_{20}H_{40}$	280.50
54	0.47	29.799	1,2,3,4,5,6,7,8-octahydro-1,1,4,4,5,5,8,8-octamethyl-anthracene	$C_{22}H_{34}$	298.50
55	0.50	29.928	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.50
56	1.02	30.069	Methyl palmitate	$C_{17}H_{34}O_2$	270.45
57	1.31	30.527	Dibutyl phthalate	$C_{16}H_{22}O_4$	278.34
58	0.60	30.886	3,6,6-trimethyl-1-(phthalazin-1-yl)-1,5,6,7-tetrahydro-4H-indazol-4-one	$C_{18}H_{18}N_4O$	306.40
59	3.74	31.033	Oleic Acid	$C_{18}H_{34}O_2$	282.47
60	1.43	31.551	Methyl octadeca-9,12-dienoate	$C_{19}H_{34}O_2$	294.47
61	1.03	32.181	1-Docosene	$C_{22}H_{44}$	308.60
62	1.08	33.375	9-Tricosene, (Z)-	$C_{23}H_{46}$	322.60
63	0.20	33.550	Hexacosanoic acid	$C_{26}H_{52}O_2$	396.70
64	0.36	34.116	Lauryl myristate	$C_{26}H_{52}O_2$	396.69

Legend: % Conc. = Percentage composition; RT = Retention time; S/N = Serial number; CCCE = *Cymbopogon citratus* chloroform extract

Table IV: Molecular docking score (Kcal/mol) of identified compounds and standard agent (sarin) against acetylcholinesterase

BINDING AFFINITY	4PQE	Hydrogen bond/distance
Carvomenthol (Kcal/mol)	-6.5	SER A:293 (2.27)
(-)-Carvone (Kcal/mol)	-6.6	PHE A:295 (2.36)
Cyclohexadecane Kcal/mol)	-8.6	-
Decalin (Kcal/mol)	-6.5	-
Eucalyptol (Kcal/mol)	-6.3	TYR A:337 (1.78)
Hemellitol (Kcal/mol)	-6.6	-
Diazinon (Kcal/mol)	-7.5	THR A:83 (4.44); TYR A:124 (2.10)

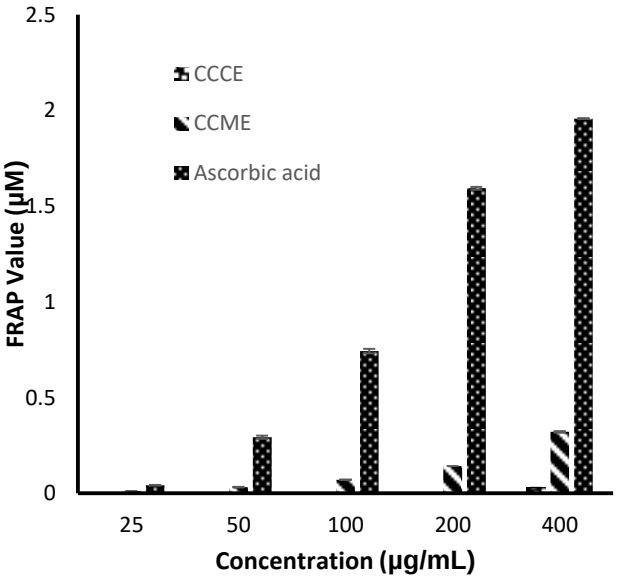

Figure II: The FRAP values of CCCE and CCME.
Legend: CCME; *Cymbopogon citratus* methanol extract, CCCE; *Cymbopogon citratus* chloroform extract, FRAP; ferric reducing antioxidant

DISCUSSION

This study investigated the phytochemical composition, antioxidant and acaricidal properties of methanol and chloroform extracts of *C. citratus* against *A. variegatus* ticks. The methanol extract produced higher antioxidant and acaricidal properties when compared to the chloroform extract. The difference in the magnitude of their pharmacological activities (antioxidant and acaricidal) could be linked to the difference in their phytochemical composition, which could be associated with the polarity of solvents used for the extraction (Shezryna *et al*., 2020). The methanol extract had higher concentration of essential oils than the chloroform extract. Essential oils are mainly extracted via hydrodistillation and thus require highly polar solvent like methanol (Shezryna *et al*., 2020). *Cymbopogon citratus* is known to be a rich source of essential oils

4PQE

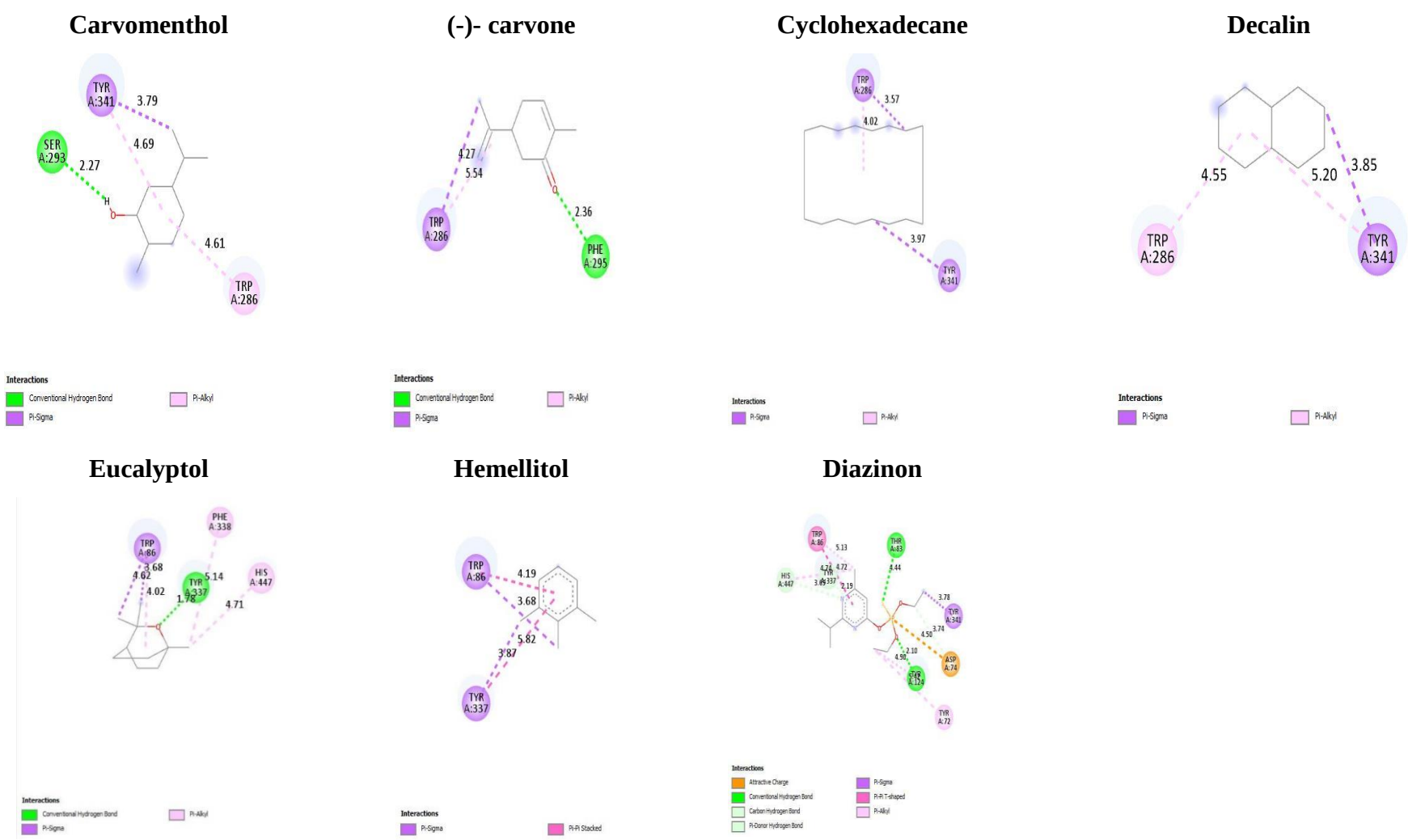

Figure III: 2D-molecular interaction of compounds, standard agent (diazinon) with retrieved target protein structure from the protein data bank, comparing active site amino acid interactions by using BIOVIA-DSV for visualization

and other bioactive compounds (Majewska *et al*., 2019). The difference in the concentration of the essential oils in this study and previously reported concentrations could be attributed to the variation in extraction method and source of the plant collection (Chiasson *et al*., 2001). In this study, methanol and Soxhlet Apparatus was used, while previous studies used water and distillation method for the extraction (Shezryna *et al*., 2020)

The extracts produced time-dependent increase in acaricidal property, which was in agreement with the report of Shezryna *et al*. (2020). The methanol extract produced higher ($P < 0.05$) acaricidal activity when compared with the chloroform extract. This could be attributed to the higher concentration of essential oil and other bioactive compounds in the methanol extract when compared with the chloroform extract. Essential oils such as carvomenthol, (-)-carvone, hemellitol, eucalyptol, cyclohexane and carane have been reported to possess cytotoxic and acaricidal properties, via the inhibition of acetylcholinesterase (Benelli and Pavela, 2018; de Oliveira et al., 2019). The finding of this study is in agreement with the report of Shezryna *et al*. (2020).

The extracts produced concentration-dependent increase in antioxidant activity. The methanol extract produced higher antioxidant activity when compared with the chloroform extract and this corroborated the higher composition of essential oils and some bioactive compounds in the methanol extract when compared with the chloroform extract. The antioxidant activity of essential oils have been reported (Tit and Bungau, 2023). The antioxidant property of the extract could be helpful in the management of stress and dermatitis associated with tick infestation in animal. The anti-stress and anti-inflammatory properties of antioxidants have been documented (Buemann *et al*., 2021).

Molecular docking analysis, which is an artificial intelligence program, is widely employed in predicting ligand interactions with receptor proteins. It is employed in drug discovery, where it measures binding affinity (docking score) (Agwupuye *et al*., 2021; Oyedemi *et al*., 2021). In this study, the studied compounds showed strong binding affinity with acetylcholinesterase that was used as the protein. The result of this study suggests the ability of the studied compounds to interact strongly with acetylcholinesterase. Carvomenthol, (-)-carvone, and eucalyptol formed hydrogen bonds with various amino acids on the protein. The ability of the studied compounds to bind the acetylcholinesterase suggests that they have the potential to inhibit the enzyme and their acaricidal property could be via the inhibition of acetylcholinesterase. Generally, one mechanism employed by acaricides is the inhibition of the enzyme acetylcholinesterase, which is critical to the tick's central nervous system (Temeyer, 2018). The enzyme acetylcholinesterase catalyzes the metabolism of acetylcholine at the synaptic cleft and its inhibition causes the accumulation of acetylcholine at the synaptic cleft and resultant paralysis of the affected organism (Temeyer, 2018). One commonly used compound for the inhibition of acetylcholinesterase is diazinon. Diazinon is an organophosphate pesticide used to control pests in both crops and animals and act via the inhibition of acetylcholinesterase (Mdeni *et al*., 2022).

In conclusion; the methanol extract demonstrated better antioxidant and acaricidal properties than the chloroform extract, which could be linked to the higher concentration of essential oils (Carvomenthol, (-)-carvone, and eucalyptol) in the methanol extract. The molecular docking revealed that the mechanism of the acaricidal property could be via inhibition of acetylcholinesterase and paralysis.

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CONFLICT OF INTEREST DECLARATIONS

The authors declare no conflict of interest

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