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IFE JOURNAL OF SCIENCE AND TECHNOLOGY

Vol 7. No. 1 (2023) 64–76

Chemical compositions, antioxidant potentials and enzyme inhibitory activities of essential oil and solvent extracts of *Anchomane difformis* (Blume) Engl. leaf

O. S. Balogun [1], O. S. Ajayi [1], T, Mudathir [1] and L. Zhiqiang [2]

[1] Department of Chemistry, Obafemi Awolowo University, Ile-Ife, Nigeria

[2] Centre for Mass Spectrometry, Changchun Institutes of Applied Chemistry, CAS, Changchun, China

*Corresponding author: balogunolaoye@oauife.edu.ng; solomonoye@gmail.com
Tel.: +234-8034940850

Abstract

Leaves of *Anchomane difformis*, an herbaceous medicinal plant commonly used in west tropical Africa for treatment of diabetes were collected, extracted with aqueous methanol to give crude methanol extract which was fractionated with *n*-hexane. Also, the volatile oil of the leaf was extracted by hydro-distillation method. The fractionated extracts, derivatised by silylation, and the volatile oil were separately analysed using GC-MS. The total phenolic content (TPC), total flavonoid content (TFC) and total antioxidant capacity (TAC) of each extract were estimated using standard procedures. Also, ferric reducing antioxidant power (FRAP) and DPPH radical scavenging assays were used to evaluate the antioxidant potential of the extracts while the enzyme inhibitory activities were determined using alpha glucosidase and porcine pancreatic lipase enzymes. Phytol was found to be predominant in all the extracts at 42.69%, 11.13% and 5.78% for the *n*-hexane, defatted and volatile oil extracts respectively. Fatty acid derivatives were the major components of the extract fractions while 1-octen-3-ol (19.02%), α-ionone (5.95%), 1,3-di-*n*-propy ladamantane (5.08%) 3,5.5-trimethyl hex-2-ene (5.01%), hexahydrofarnesyl acetone (4.46%) and trans-geranylacetone (4.24%) were the principal constituents of the volatile oil. The crude had highest TFC (46.43 ± 5.34 mg QuE/g), TAC (81.35 ± 9.61 mg AAE/g) and FRAP (40.36 ± 5.01 mg AAE/g) while the volatile oil scavenged DPPH free radical better than other extracts. All the extracts inhibited alpha glucosidase enzyme and none showed inhibition porcine pancreatic lipase enzymes.

***Keywords*:** Essential oil; phytol; hypoglycemic activity; phenolic profile; antidiabetic

Introduction

Anchomanes difformis is a rhizomatous herbaceous plant in the family Araceae that is endemic in tropical West African forest. It is an important medicinal plant used in Nigeria to manage respiratory diseases, cough, throat sore and diabetes (Akah and Njike, 1990; Ahmed, 2018). Specifically, traditional herbal practitioners of Udu and Ughievwen clans of Delta State, Nigeria use the decoction of the plant extensively for treatment of asthma (Idu *et al*., 2003). In Guinea, the rhizome is used as rubefacient while it serves as purgative

agent, poison antidote and diuretics in Ivory Coast (Adeyemi *et al*., 2015). The presence of secondary metabolites such as saponins, tannins, alkaloids amino acids and heterosides have been reported in the leaf, stem and rhizome of the plant (Odeghe *et al.*, 2020). However, despite the enormous ethno-medicinal relevance of *A. differmis*, the chemical constituents and bioactivities of its essential oil are yet to be reported. Here in, we report the chemical composition of essential oils and solvent extracts of leaf of *A. differmis*, and also evaluated their antioxidant activities and inhibition of porcine lipase and alpha-glucosidase enzymes.

Materials and Methods

Plant Collection and Preparation

Leaves of *A. differmis* were harvested from a forest in Osengere village, Ibadan, Nigeria and identified by in the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Nigeria. The leaves were dried at laboratory ambient temperature and were pulverized thereafter. The solvent extracts were prepared by soaking the leaves (500 g) in aqueous methanol (95%) to give a crude extract (ADLC) (62.0 g) which was partitioned with *n*-hexane to give 23.0 g *n*-hexane fraction (ADLH) and the remaining 37.0 g defatted fraction (ADLD).

The essential oil (ADLE) was obtained by hydro-distilling of the pulverized leaves (500 g) using Clevenger apparatus. The volatile oil extracted was dried over anhydrous Na_2SO_4, stored in air-tight amber bottles until analysis.

Extract Silylation

Five milligram each of ADLH and ADLD were separately dissolved in dry pyridine (50 µL) and heated at 80 °C for 20 min with 60 µL silylating agent, N, O-bis-(trimethylsilyl) trifluoroacetamide containing 1% trimethylchlorosilane. The silylated products were thereafter dissolved in chloroform and analysed on TSQ 8000 Evo GC (Thermo Scientific) system with a Mass Selective Detector (MSD).

GC/MS Analysis

The extracts were analysed on TSQ 8000 Evo GC (Thermo Scientific) system with a Mass Selective Detector (MSD). The instrument had a HP-5MS (5% Phenyl methyl siloxane) capillary column (length 30 m, inner diameter, 0.25 mm and film thickness 0.25 µm). In the analysis of essential oil, the initial GC oven temperature was 40 ^{o}C for 2 min and was taken through three successive ramps. First ramp was set at 5.0 ^{o}C/min to a final temperature of 110.0 ^{o}C with 3 min hold time, second ramp was maintained at 1.0 ^{o}C/min to final temperature of 140.0 ^{o}C, with hold time of 3 min while third ramp was put at 5.0 ^{o}C/min to a final temperature of 300.0 ^{o}C with 3 min hold time. The carrier gas was helium gas, at flow rate of 1.00 mL/min and the sample (0.5 µL) was injected in the splitless mode. The following conditions were used for the silylated extract: The initial GC oven temperature

was 40 °C for 1 min and was taken through two successive ramps. Ramp 1 was put at 15.0 °C/min to a final temperature of 180.0 °C, with 1 min hold time while ramp 2 was maintained at 5.0 °C/min to final temperature of 300.0 °C, with hold time of 8 mins. The carrier gas also helium gas, at flow rate of 1.00 mL/min and the sample (1.0 µL) was injected in the splitless mode. The splitless flow and splitless time were 33.3 mL/min and 1.0 min respectively for the analysis.

The GC was coupled to MS transfer line heater set at 250 ^{o}C with EI (positive) ionisation mode. The mass range was 30-600 amu at scan time of 0.2 s. Qualitative identification of different constituents was based on comparisons of the relative retention indices and mass spectra with those of the Wiley and NIST library of the GC/MS through the use of probability merge search software and the NIST MS spectra search program. In some cases, further comparisons were made using retention indices and mass spectra reported in literature (Adam, 1989). The relative amount (% composition) of individual component of the oil was expressed as percentages of the peak area relative to the total peak area.

Determination of Total Phenolic Content (TPC)

The total phenolic contents of the solvent extracts were determined using Folin–Ciocalteu reagents as described by Slinkard and Singleton (1977) with slight modifications. 1 mL aliquot of each extract was reacted with 200 µL of Folin-Ciocalteu reagent, 5 mins later, 1 mL of 7% Na_2CO_3 was added and the resulting mixture was incubated in dark at room temperature for 90 min. The absorbance against negative control containing distilled water in place of plant extract was taken at 750 nm and TPCs were estimated using gallic acid calibration curve. The results were expressed as gallic acid equivalents (GAE) mg/g of plant extract (Ghasemzadeh *et al*., 2010)

Determination of Total Flavonoid Content (TFC)

A modified method (Bushra *et al*., 2009) was used to determine the TFCs of the solvent extracts. Briefly, 0.5 mL aliquot of varying concentrations of each extract (0.1 - 0.5 mg/mL) was mixed 5% (w/w) $NaNO_2$ (0.1 mL), after 5 min, 0.1 mL of 10 % $AlCl_3$ and 0.2 mL of 1.0 M NaOH were added and the volume was made up to 2.5 mL with distilled water. The absorbance against negative control containing distilled water in place of plant extract was taken at 510 nm and TFCs were estimated using quercetin calibration curve. The results were expressed in mg quercetin/g of plant extract

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacities of the solvent extracts were evaluated by the method of Prieto *et al*. (1999). The method is premised on the reduction of molybdenum (VI) to molybdenum (V) by the extract and subsequent formation of a green phosphate molybdenum (V) complex. An aliquot of 100 µL of the extract and standard solution of ascorbic acid (20 - 100 µg/mL) were added to 1 mL of the reagent solution containing 0.6

M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The resulting solution was incubated at 95 ºC for 90 min and absorbance was taken at 695 nm against blank after cooling to room temperature. The total antioxidant capacity was expressed as the number of gram equivalent of ascorbic acid.

Determination of Ferric Reducing Antioxidant Power (FRAP)

This assay was carried out using the method described by Benzie and strain (1996). One milliliter of the FRAP reagent was mixed with a 50 µL aliquot of each of the solvent extracts at 0.1 mg/mL and standard solutions of ascorbic acid (20 -100 µg/mL). The mixture was thereafter incubated at 37 ºC for 30 min in the dark and absorbance was measured against the blank at 593 nm. Standard graph was prepared using ascorbic acid solution and the results were expressed in mg of ascorbic acid equivalents (AAE)/g of extract.

A 50µL aliquot of the extract, solvent fractions, and the isolated compounds at 0.1 mg/mL and 50 µL of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/mL) was added to 1 mL of FRAP reagent. Absorbance measurement was taken at 593 nm exactly 10 minutes after mixing against reagent blank containing 50 µL of distilled water.

DPPH Radical Scavenging Assay

The method described by Brand-Williams *et al*. (1995) was used. To 1 mL of varying concentration (0.3125 - 10 mg/mL) of the solvent extracts and the essential oil, 1 mL of methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (0.3 Mm DPPH) was added and the mixture was incubated in dark at room temperature for 30 min. The absorbance was measured against the blank at 517 nm and the percentage inhibition was calculated using the equation:

$$Inhibition\ (\%) = \frac{OD\ control - OD\ sample}{OD\ control} \times 100 \qquad (1)$$

Lipase Inhibition Assay

The pancreatic lipase inhibitory assay was performed using method described by Balogun *et al*., (2020). Porcine pancreatic lipase (0.50 mg/mL) and 4.5 mM *p*-nitrophenyl laurate (containing 1% Triton X-100) were prepared in Tris-HCl buffer solution. A 40 µL aliquot each of porcine pancreatic lipase, buffer and varying concentrations (12.5 - 100 µg/mL) of ADLH, ADLM and ADLE were added in 96-well plate and thereafter incubated at 37 ºC for 5 min. After pre-incubation, 80 µL of *p*-nitrophenyl laurate was added to start the reaction. The mixtures were incubated at 37 ºC and after 30 min the reaction was stopped using 100 µL sodium acetate buffer (pH 4.2). The absorbance was taken at 405 nm on Tecan GENios multifunctional microplate reader (Männedorf, Switzerland). Buffer solution and orlistat were used in place of sample as positive and negative controls respectively. The experiment was performed in triplicates and the inhibition (%) was calculated using

equation (1).

α-Glucosidase Inhibition Assay

A 40 µL aliquot each of varying concentration (12.5 - 100 µg/mL) of ADLH, ADLM and ADLE were incubated for 5 min at 37 °C with 40 µL of 0.2 U/mL α-glucosidase in phosphate buffer and 20 µL of 100 mM. phosphate buffer (pH 6.8). The reaction was initiated with 100 µL of 2.5 mM *p*-nitrophenyl-α-*D* glucopyranoside in phosphate buffer incubated further for 30 min. Thereafter, 100 µL of sodiumcarbonate (0.2 M) was used to terminate the reaction and the absorbance was taken at 405 nm on the microplate reader. The positive and negative controls were phosphate buffer solution and acarbose respectively. The inhibition (%) was calculated using equation (1).

Results and Discussion

The GC-MS analysis of silylated ADLH indicated presence of nineteen compounds (Fig 1) in which phytol (42.69 %), stearic acid (14.71 %) and palmitic acid (14.18 %) were the principal constituents (Table 1). Phytol is a diterpenoid which has been reported to exhibit broad spectrum of biological activities such as antioxidative, cytotoxity, anti-inflammatory and antimicrobial (Prabuseenivasan *et al*., 2006; Murbach *et al*., 2014; Silva *et al*., 2014). Its antioxidant properties have been attributed to a radical scavenging mechanism involving the allylic alcohol (Islam *et al*., 2016). Similarly, the silylated defatted fraction contained 21 compounds (Fig. 2), of which 6-hydroxynorleucine (19.02 %) and phytol (11.13 %) were highest in composition (Table 2). Also, the analysis of the essential oil indicated presence of 44 compounds which were mostly terpenoids and non-isoprenoid hydrocarbons of which 1-octen-3-ol (19.02%), α-ionone (5.95%), phytol (5.78%), 1,3-di-*n*-propy ladamantane (5.08%), 3,5.5-trimethyl hex-2-ene (5.01%), hexahydrofarnesyl acetone (4.46%) and trans-geranylacetone (4.24%) were predominant (Fig 3, Table 3). Okull *et al*., (2003) demonstrated the inhibition of a common food spoilage organism, *Penicillium expansum* PP497A, using 1-octen-3-ol at concentration 160 µg/g in weakly acidic medium which suggested that volatile oil rich in 1-octen-3-ol could serve as a food preservative and anti-fungal agent. In a similar report, fumigation of *Monilinia fructicola* infected peach fruit with 1-octen-3-ol showed inhibition of the pathogen and decrease in decay rate (Wang *et al*., 2022). Also, α-ionone and its derivatives have been shown to exhibit antioxidant, anti-inflammatory, antimicrobial and anticancer effects (Aloum *et al*., 2020; Huang *et al*., 2022). Furthermore, α-ionone, geranyl acetone and phytol which were the major terpenoidal constituent of the essential oil are well reputed for biological activities such as antioxidant, anti-trypanosomal and anticancer (Bero *et al*., 2013; Stobiecka, 2015; Saad *et al*., 2019).

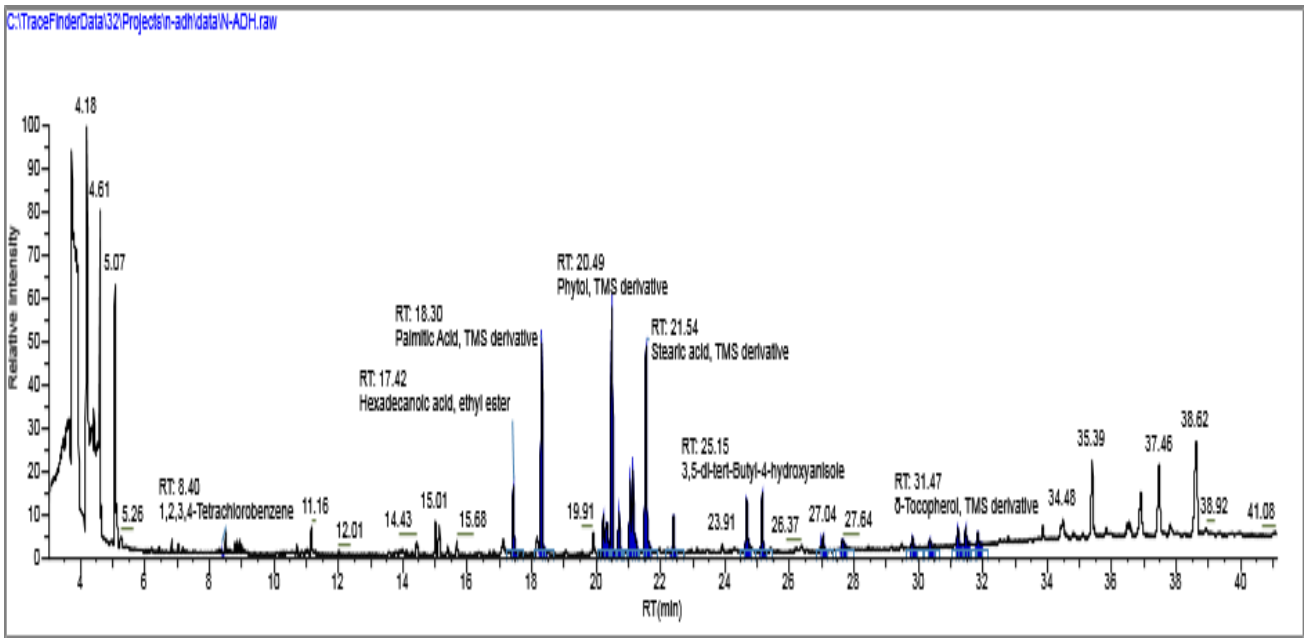

Figure 1: GC chromatogram of *n*-hexane fraction of *A. differmis* leaf

Table 1: Chemical Constituents of *n*-Hexane Fraction of *A. Differmis* Leaf

	Chemical Constituent	RT (min)	% Composition
1.	Hexadecanoic acid ethyl ester	17.42	4.68
2.	Palmitic acid	18.3	14.18
3.	n-Propyl 9,12-octadecadienoate	20.22	1.18
4.	(E)-9-Octadecenoic acid ethyl ester	20.31	0.71
5.	Phytol	20.49	42.69
6.	Ethyl 13-methyl-tetradecanoate	20.7	2.61
7.	9,12-Octadecadienoic acid	21.05	1.54
8.	Oleic acid	21.13	2.34
9.	Stearic acid	21.54	14.71
10.	2,4-Dodecadienoic acid, 11-methoxy-3,7,11-trimethyl-, methyl ester, (E,E)-	22.4	1.36
11.	Arachidic acid	24.66	3.41
12.	3,5-di-tert-Butyl-4-hydroxyanisole	25.15	5.53
13.	1-Monopalmitin	27.04	0.74
14.	Heptadecanoic acid	27.65	0.78
15.	Glycerol monostearate	29.81	0.49
16.	C(14a)-Homo-27-norgammacer-13-en-21-one, 3-methoxy-, (3α)-	30.35	0.54
17.	6,6-Diethylhoctadecane	31.22	0.73
18.	δ-Tocopherol	31.47	1.12
19.	Hexacosanoic acid ester	31.84	0.67
	Total		**98.98**

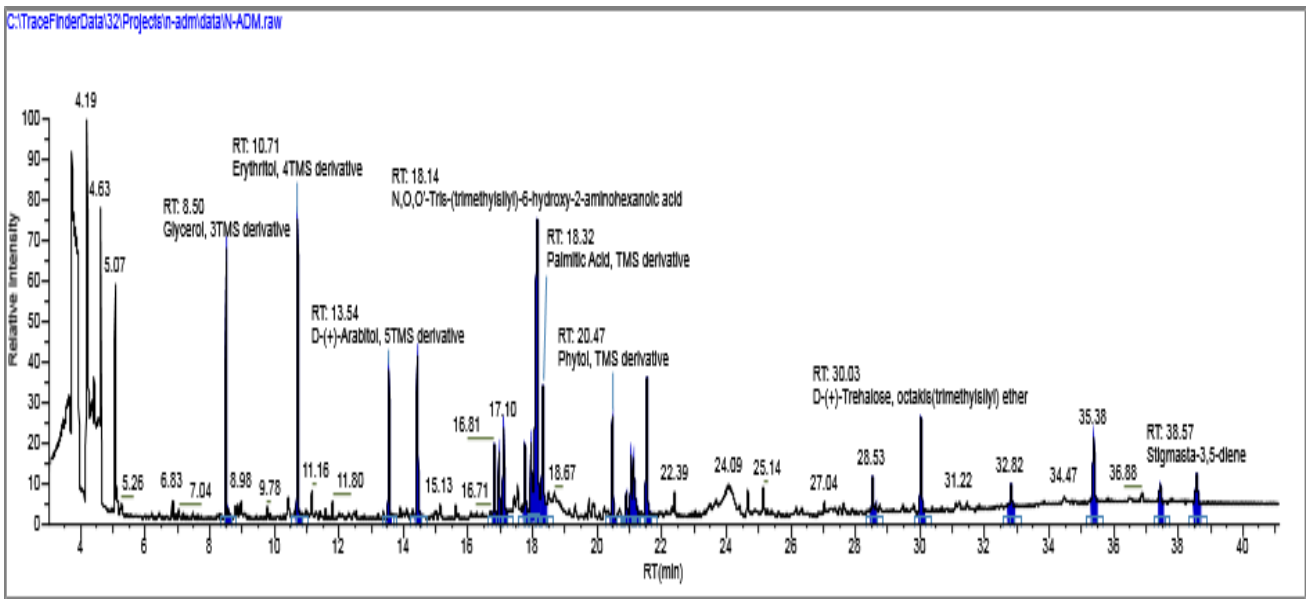

Figure 2: GC chromatogram of defatted fraction of *A. differmis* leaf

Table 2: Chemical Constituents of Defatted Leaf Extract of *A. Differmis*

	Chemical constituent	RT (min)	% Composition
1.	Glycerol	8.5	8.62
2.	Erythritol	10.71	8.72
3.	D-(+)-Arabitol	13.54	6.44
4.	D-Ribofuranose	14.43	7.65
5.	1,5-dioxaspiro[5.5]undecane-3-carboxylic acid	16.81	2.83
6.	D-Mannitol	16.96	2.08
7.	9,10-anthracenedione, 1,5-dihydroxy-4,8-bis[(4-methylphenyl)amino]-	17.1	3.10
8.	β-Arabinopyranose	17.76	2.13
9.	Altronic acid, γ-lactone	17.95	1.99
10	6-Hydroxynorleucine	18.14	19.02
11	Palmitic Acid	18.32	5.65
12	Phytol	20.47	11.13
13	D-Xylofuranose	20.91	0.82
14	9,12-Octadecadienoic acid	21.05	0.76
15	Oleic Acid	21.13	1.38
16	Stearic acid	21.53	6.29
17	Sucrose	28.53	1.70
18	D-(+)-Trehalose, octakis(trimethylsilyl) ether	30.03	5.71
19	Allonic acid, γ-lactone	32.82	0.84

2(	3α,5α-Cyclo-ergosta-7,9(11),22t-triene-6β-ol	37.45	0.22
2	Stigmasta-3,5-diene	38.57	0.39
	Total		**97.47**

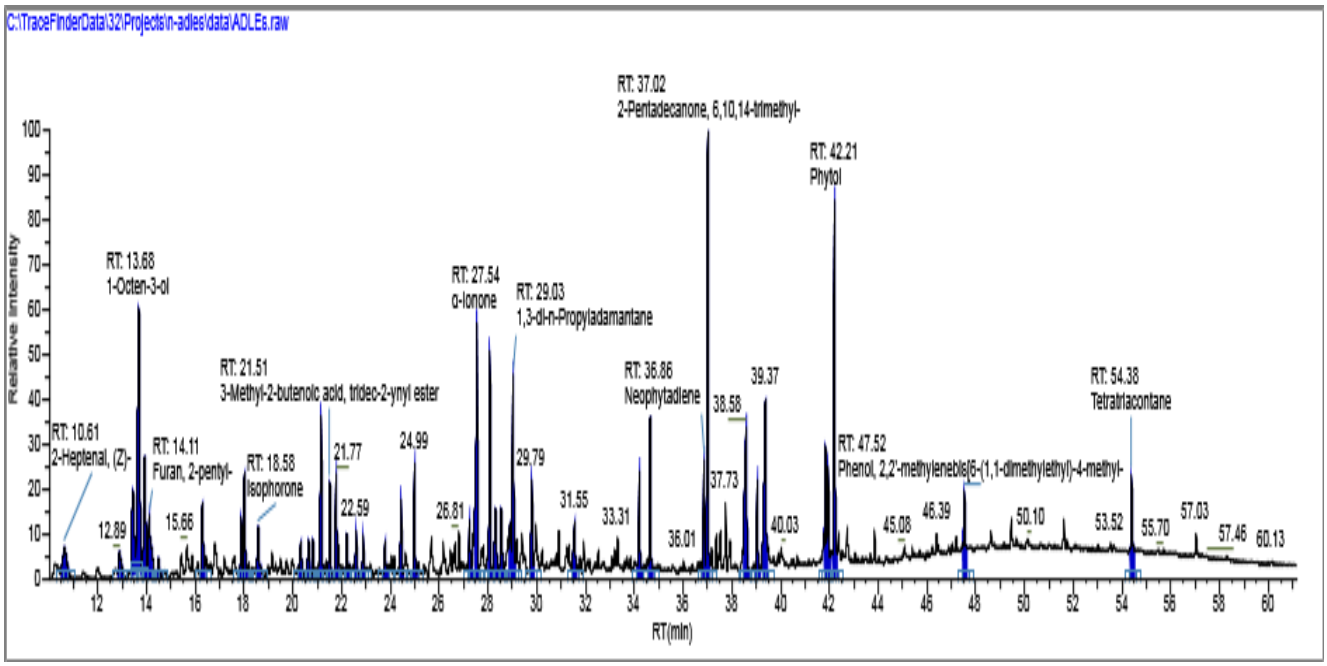

Figure 3: GC chromatogram of essential oil of *A. difformis* leaf

Table 3: Chemical Constituents of Essential Oil of *A. Difformis* Leaf

	Chemical constituent	RT (min)	% Composition
1.	2-Heptenal	10.61	0.89
2.	Benzaldehyde	12.89	1.36
3.	3,5,5-Trimethyl-2-hexene	13.41	5.01
4.	1-Octen-3-ol	13.68	19.02
5.	Sulcatone	13.91	2.93
6.	2-Amylfuran	14.11	3.43
7.	2-Octen-1-ol	14.48	0.19
8.	2,4,4-Trimethyl-2-cyclohexen-1-ol	16.28	1.22
9.	Linalool	17.87	0.88
10.	3,5,5-trimethyl-2-Cyclopenten-1-one	18.03	2.45
11.	Isophorone	18.58	2.67
12.	2,6-Dimethylbenzaldehyde	20.32	0.83
13.	1-[2-(Hydroxymethyl)phenyl]ethano	20.62	0.71
14.	2-Decanone	20.81	0.78
15.	1,3-Dimethyl-5-ethyladamantane	21.15	2.63
16.	3-Methyl-2-butenoic acid, tridec-2-ynyl ester	21.51	3.58

17.	β-Cyclocitral	21.77	1.19
18.	p-Menth-4-en-3-one	22.59	0.64
19.	β-Homocyclocitral	22.87	0.43
20.	1-Cyclopropyl-1-methyl-ethylamine	23.78	0.52
21.	(-)-Car-3-en-2-one	24.43	0.90
22.	β-Cyclohomogeraniol	24.99	1.12
23.	β-Ionone	27.24	0.72
24.	α-Ionone	27.54	5.95
25.	*trans*-Geranylacetone	28.07	4.24
26.	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexahydro-gamma-chromene	28.3	1.56
27.	epi-β-Caryophyllene	28.53	0.40
28.	α-Curcumene	28.91	0.51
29.	1,3-di-n-Propyladamantane	29.03	5.08
30.	Cyclohexanecarboxylic acid, 3-fluorophenyl ester	29.8	2.45
31.	Caryophyllene oxide	31.56	0.31
32.	Eicosanal	34.2	0.84
33.	2-isoHexyl-6-methyl-1-heptene	34.64	1.52
34.	Neophytadiene	36.86	1.00
35.	Hexahydrofarnesyl acetone	37.02	4.46
36.	Farnesyl acetone	38.52	1.68
37.	Hexadecanoic acid, methyl ester	38.58	2.57
38.	3,5,11,15-Tetramethyl-1-hexadecen-3-ol	39.05	2.58
39.	Ascorbic acid dipalmitate	39.37	1.85
40.	(9Z,11E)-Octadecadienoate	41.84	0.91
41.	cis-13-Octadecenoic acid, methyl ester	41.94	0.44
42.	Phytol	42.21	5.78
43.	*p*-Cresol	47.52	0.86
44.	Tetratriacontane	54.38	0.90
	Total		99.99

The polyphenolic profile of ADLC showed it had flavonoid content and antioxidant capacity of 46.43 ± 5.34 mg QuE/g and 81.35 ± 9.61 mg AAE/g respectively which were higher than the fractioned extracts (ADLH and ADLD). However, its phenolic content (10.58 ± 2.50 mg GAE/g) is lower compared to ADLD (13.68 ± 0.60 mg GAE/g) this might due to the differential distribution of bound and free phenolics in the partitioning solvents used (Table 4). Comparatively, phenolic profile of the crude extract was better than those of the partitioned fractions. Previous studies on polyphenolic contents of leaf of *A. differmis* showed a range of 6.12 -26.34 for TPC (Guetchueng *et al*., 2015; Faleye *et al*., 2018; Bello *et al*., 2020; Kouassi *et al*., 2022) while 49.13 ± 0.51 mg REs/g was reported as TFC by Bello *et al*., (2020). Similarly, the total antioxidant capacity was highest in ADLC (81.35 ± 9.61 mg AAE/g) compared to the fractionated extracts. The TAC value was also, much higher than those reported by Aliyu *et al*., (2013) which ranged from 3.36-3.81 mg

AAE/g for different solvent extracts of leaf of *A. differmis.*

The antioxidant potentials of the extracts and the essential oil were evaluated using FRAP and DPPH radical scavenging assays. In both experiments, the crude extract demonstrated highest reducing (40.36 ± 5.01 mg AAE/g) and DPPH free radical scavenging ability (5.47 ± 1.05 mg/mL) among the solvent extracts, followed by the deffated fraction (34.86 ± 2.55 mg AAE/g and 6.51 ± 0.98 mg/mL) which were much lower than standard ascorbic acid (0.008 ± 0.00 mg AAE/g) (Table 4). The result showed a good nexus between the observed

Table 4: Antioxidant Activities of Extracts of *A. Differmis*

	TPC (mg GAE/g)	TFC (mg QuE/g)	TAC (mg AAE/g)	FRAP (mg AAE/g)	DPPH IC_{50} (mg/mL)
ADLC	10.58 ± 2.50	46.43 ± 5.34	81.35 ± 9.61	40.36 ± 5.01	5.47 ± 1.05
ADLH	0.682 ± 0.363	2.635 ± 0.272	16.084 ± 5.108	7.30 ± 0.85	6.51 ± 0.98
ADLD	13.68 ± 0.60	16.873 ± 0.697	22.365 ± 6.277	34.86 ± 2.55	2.09 ± 0.31
ADLE	NT	NT	NT	NT	0.121 ± 0.002
Ascorbic acid					0.008 ± 0.00

NT: Not tested. Data are expressed as means ± SD (n = 3).

polyphenolic content and antioxidative potential of the extracts (Table 5). The solvent extracts inhibited alpha glucosidase enzyme better than the essential oil (ADLE) and the standard but had very poor inhibitory activity on the porcine pancreatic lipase while the essential oil showed no activity on the lipase enzyme. Investigations of Bello *et al*., (2020) on underutilized Nigerian vegetables indicated that *A, differmis* exhibited a significantly higher antioxidant activity than other vegetables, and also moderately inhibited α-glucosidase.

Table 5: Enzyme inhibitory Activities of Extracts of *A. Differmis*

	Glucosidase IC_{50} (µg/mL)	**Lipase (µg/mL)**
ADLC	56.13 ± 2.58	31.49 ± 3.73
ADLH	49.05 ± 2.56	47.75 ± 2.62
ADLE	122.51 ± 6.99	NA
CONTROL	279 ± 4.21	0.88 ± 0.12

NA: No activity

Conclusions

In conclusion, leaf extract of *A. differmis* has a rich polyphenolic content, demonstrated good antioxidant properties, and may find application in the management of diabetic conditions as it is being currently used in folkloric medicine.

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