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Original Research Article

The Volatile Constituents from *Lepidium sativum* and *Tridax procumbens* Shoots Exhibit Antimicrobial Effect and Inhibitory Activities Against α-Amylase Enzyme and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical

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ABSTRACT

The bioprospecting of wild functional food plants used in the urban communities in developing countries as sources of food and medicine has been on the rise steadily. *Lepidium sativum* and *Tridax procumbens* are wild edible plants in Nigeria with tremendous ethnomedicinal uses. In this research, volatile extracts were obtained from the shoots of the two plants via steam distillation and subjected to Gas Chromatography Mass Spectrometry analysis, antimicrobial analysis and inhibitory activities against α-amylase enzyme and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, respectively. A white color volatile extract was obtained from *L. sativum* shoot while a translucent volatile extract with sweet scent was obtained from *T. procumbens* shoot. Both extracts consist of vary constituents of phytochemicals. The extract from *L. sativum* only showed moderately susceptibility against *Streptococcus pneumonea* with zone of inhibition of 11.5 mm among the selected microbes while extract from *T. procumbens* showed a broader susceptibility towards more microbes with higher zone of inhibition values and a better inhibitory activity against *Staphylococcus aureus* with minimum inhibition concentration MIC of 31.23 mg/g. Both extracts have better alpha-amylase inhibitory activity with EC_{50} values of 134.48 µg/mL (*L. sativum*) and 106.74 µg/mL (*T. procumbens*), respectively when compared to the EC_{50} value of 165.93 µg/mL of the standard drug acarbose. However, the standard drug (ascorbic acid) exhibited better inhibitory activity against DPPH radical with EC_{50} value of 41.69 µg/mL when compared to their EC_{50} values of 47.71 and 48.63 µg/mL, respectively. The results show that the volatile extracts contain phytochemicals with antimicrobial, anti-diabetic and antioxidant potentials.

Keywords: *Lepidium sativum*, *Tridax procumbens,* Volatile extracts, Gas Chromatography Mass Spectrometry analysis, α-Amylase enzyme, 2,2-Diphenyl-1-picrylhydrazyl radical, Inhibitory activity

Introduction

Lepidium sativum Linn. is a vegetable plant known as the 'garden cress' and belong to the family of Brassicaceae, and genus of *Lepidium*. It is widely distributed in many countries around the world, virtually in all the continents except Antarctica. In Africa, it is predominantly found in the Northern and Eastern parts of Africa; in countries such as Egypt, Ethiopia and Kenya.[1] It is also reportedly found in the Western part of Africa; in countries like Mali and Nigeria. In Nigeria, it grows particularly in the North-Western parts of the country where it is called '*lansir*' or '*laussur*' among the people of the region and eaten raw like vegetables such as lettuce or cooked like spinach.[2, 3]

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The plant is used extensively in traditional medicines for treatment of respiratory tract disease such as asthma, bronchitis, cough, chest pain and pneumonia and as well as in the treatment of dysentery and stomachache.[4, 5]
The seeds are used as diuretic, tonic, demulcent and carminative, also to cure throat diseases, uterine tumor, nasal polyps and breast cancer.[1] Literature search have shown that extensive research has been done on the seeds of the plant in comparison to other parts of the plant. The oil and extracts from the seeds are proven to have various pharmacological activities such as wound healing and anti-inflammatory effect that causes a significant reduction of wound diameter and a significant decline of TNF-α and MMP-9 levels in skin homogenate.[6] The seed extracts were also reported to have antimicrobial activities against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Klebsiella pneumoniae,* and *Candida albicans*.[7, 8, 9] Antioxidant activity[9] and anti-trypanocidal activity against *Trypanosoma evansi*.[10] Cytotoxicity effect against Jurkat E6-1 cancer cells[11] was also reported and as well as a potent hypoglycemic effect that suppresses blood glucose, cholesterol, triglyceride, and urea level in diabetic rats.[12] Additionally, organic extract from the leaves of the plant also showed cytotoxicity effect and antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae*, *Candida albicans* and *Aspergillus flavus*.[13]

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Tridax procumbens Linn. known as *"coat buttons"* or *'Tridax Daisy"* is also called *"Ghamra"* in *Hindi, "Chiravanak*" in Malayalam, "*Herbe Caille*" in French,[14] "*Harantama*" or "*Muhibba*" in Hausa and "*Igbalode*" in Yoruba.[15] The plant is a perennial plant from the Asteraceae family which is considered as functional food and the leaves are cooked and eaten as vegetable.[16] It is native to tropical America, Africa, Australia and Asia.[14] *T. procumbens* has been well documented used in folk medicines for treatment of many ailments including anemia, colds, typhoid fever, cough, stomach pain, diarrhea, diabetes, epilepsy, back ache, skin infections, inflammation and wound healing.[17] Extracts (organic and aqueous) and essential oils from leaves of the plants exhibited antimicrobial activities against different microbial organisms such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis*, *Klebsiella pneumoniae, Salmonella typhi*, *Proteus mirabilis*, *Aspergillus niger*, *Aspergillus fumigatus* and *Penicillium chrysogenum*.[18, 19, 20] Nanoparticles synthesized from *T. procumbens* leaves using zinc oxide *(*TPE-derived ZnO NPs*)* were found to possess potent anti-diabetic activity in a streptozotocin (STZ)-induced diabetic rats.[21] Another study by Bhagwat *et al*.[22] also showed that the extracts from the leaves of the plants significantly reduced the blood glucose level in alloxin-induced diabetic rats. Other pharmacological activities the plant possesses are but not limited to anticancer activity against breast cancer cell line MCF 7[23, 24] and B16F-10 melanoma cells in C57BL/6 mice,[25] antioxidant activity[20, 26] and contain antivirus compounds including betulinic acid, kaempferol and lignin that inhibit 3CLpro receptor of SARS-CoV-2 virus in an *in silico* study.[27] Literature has indicated the efficacy of essential oils and extracts (organic and aqueous) from the plants as antimicrobial, antioxidant and antidiabetics agents. However, volatile extracts obtained from the shoots of the plants via steam distillation has not been reported. This is the first research that shows that the volatile extracts obtained from shoots of the plants via steam distillation exhibited inhibitory activities against α-amylase enzyme and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and as well as antimicrobial activity against *Streptococcus pneumonea*, *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus* which revealed their potentials as alternative sources of anti-infectious agents.

Materials and Methods

Sample collection, preparation and extraction

The *Tridax procumbens* plant was collected from farmlands at Gwiwa Low-cost area (12°58'57.6"N 5°12'21.6"E) in Sokoto state on 9th February, 2022 while *Lepidium sativum* was purchased from vegetable market (12°10'47.4"N 6°40'00.4"E) on 6th December, 2022 at Gusau Zamfara state, Nigeria. The plants were identified, authenticated and assigned the voucher numbers; FUG/BIO/HEB/2022/0081 for *T. procumbens* and FUG/BIO/HEB/2022/085 for *L. sativum*, respectively. The fresh aerial parts were cut-off, washed and drained in a plastic sieve to remove the water. About 2.35 kg of *L. sativum* and 922.9 g of *T. procumbens* were subjected independently to steam-distillation by using apparatus that consists of a round bottom solvent/boiling flask (2000 mL) that was connected to a sample/biomass flask (2000 mL). About 1000 mL of water was added to the boiling flask and placed into a heating mantle set at 60ºC for 4hrs. The steam produced passes into the sample/biomass flask containing the plant shoot and diffuses out the volatile content of the sample. The volatile content passed into the condenser (cooling unit) and were collected as mixture with water in a separation funnel. The water was removed using rotary evaporator RE-52A under vacuum and the volatile extracts were collected as white substance weighing 0.155 g from *L. sativum* labeled as LP and a sweet scent translucent substance weighing 0.554 g from *T. procumbens* labeled as TP which they gave 0.007% and 0.06% yield, respectively.

Gas Chromatography Mass Spectrometry GCMS analysis

About 1 mg/mL solution of each volatile extract was prepared using dichloromethane (DCM) as solvent. The solution was filtrated using 0.45 µm polytetrafluoroethylene (PTFE) membrane filter and 2 µL of the solution was injected into the Agilent 5977 GCMSD with Flame Ionization Detector FID. The extract was separated on Agilent (19091S-433UI) HP-5MS column of 30 m x 250 µm x 0.25 µm size using helium gas as the carrier gas at the flow rate of 1 mL/min. The heater temperature was maintained at 250ºC and transfer line temperature at 280ºC. The masses were scanned from the range of 46 – 600 amu and compounds were identified using the NIST14.L library for Mass Spectral Database.

The 1,1-diphenyl 1-2-picrylhydrazyl (DPPH) free radical scavenging activity analysis

The volatile extracts; LP and TP were examined for free radical scavenging ability against DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical using Tuba and Gulcin[28] method. The stock solutions of 1000 ppm of the volatile extracts and ascorbic acid (control) were prepared by dissolving 1 mg of the extract in 1 mL of ethanol. About 0.3 mM solution of DPPH was prepared in ethanol and 500µL (0.5 mL) of the DPPH solution was added to 1 mL of each extracts and ascorbic acid at various concentrations (10 – 150 µg/mL). The solutions were mixed thoroughly and incubated in the dark for 30 minutes at room temperature using Agilent BioTek Epoch Microplate Spectrophotometer and the absorbance was read at 517 nm against blank without extract that was only 0.5 mL DPPH solution without the extract. The free radical scavenging activities of the extracts were calculated from the Equation 1 and the EC_{50} values were determined by sigmodal dose response using logistic regression model[29] as implemented in Origin 8 software.

$$\% \text{ Inhibition} = \left(1 - \frac{As}{Ax}\right) \times 100 \quad \text{[Equation 1]}$$

As = Absorbance of the sample/ascorbic acid Ax = Absorbance of the blank

The alpha-amylase inhibitory activity analysis

The α-amylase inhibitory activities of the volatile extracts were determined according to Shai *et al.*[30] method with some modifications. Stock solutions of 1000 ppm of 5% dimethylsulfoxide DMSO extracts and acarbose were prepared, respectively. About 250 µL of each extract and that of acarbose at different concentrations (50-300 µg/mL) were incubated with 500 µL of porcine pancreatic amylase (2 µg/mL) enzyme in phosphate buffer (100 mM, pH 6.8) solution at 37ºC for 20 minutes. Then, 250 µL of 1 % starch as substrate was dissolved in to another 100 mM phosphate buffer (pH 6.8) to make a solution which was then added to the reaction mixture and further incubated at 37ºC for 1 hour. After incubation, dinitrosalicylate color reagent (1 mL) was added and allowed to incubate again for 10 minutes. The absorbance of the resulted mixture was measured at 540 nm against the blank. The percentage inhibitory activity of the extracts and acarbose were calculated using Equation 1 and the EC_{50} values were determined using Gafar *et al*.[29] method.

Antimicrobial analysis

The antimicrobial activities were carried out using three lung pathogenic bacteria; *Streptococcus pneumonea*, *Staphylococcus aureus* and *Escherichia coli* and two fungal; *Aspergillus flavus* and *Aspergillus fumigates*. The inoculum of the microbes, antimicrobial assay and minimum inhibitory concentration were performed using standard methods.

Preparation of microbial inoculums

The microbial inoculums were carried out by Atef *et al*.[31] method with some modifications; bacteria inoculums were standardized to give a density of 10^6 colony-forming units (CFU)/mL. A loopful of the test organism was inoculated into 5.0 mL of nutrient broth and incubated at 3°C for 24 hours. 0.2 mL from the 24-h cultured organism was dispensed into 20 mL sterile nutrient broth and incubated for 3–5 h to standardize the culture to 10^6 CFU/mL (corresponding to 0.5 McFarland standards). Plates were inoculated for about 15 minutes to avoid changes in inoculum density whereas the inoculum suspensions for fungal were prepared by rubbing the fungus culture with a sterile loop and collecting spores in malt extract medium followed by vortexing for 15 seconds. The inoculum suspension for spores was collected in potato dextrose broth. The suspension was cleared of residual hyphae by passing through sterile cheese cloth. The inoculum was adjusted to 1 to

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5×10^6 spores/mL by adjusting the optical density at 625 nm to between 0.08 and 0.1.

Antimicrobial assay

The antimicrobial screenings were prepared using the agar well diffusion method as described by Atef *et al*.[31]. The agar plates were prepared by spreading 100 µL of fresh microbial culture (10^6 CFU/ml) on a Muller Hilton agar plate for bacteria and on a Muller Hilton agar supplemented with 2% glucose for fungi. On the agar medium 4 wells of 6-mm diameter were created and filled with volatile extract of 100 mg/ml concentration using a micropipette under aseptic conditions. All the plates were allowed to stand for 1 hour to facilitate pre-diffusion of the extract into the medium. For the negative control DMSO was used. The plates were aerobically incubated in an upright position at 37 ± 2°C for 24 hours for bacteria and at 25 ± 2°C for 72 hours for fungi. The antimicrobial screening was evaluated by measuring the zone of inhibition (mm).

Minimum inhibitory concentration (MIC) determination by microtitre broth dilution method using p-Iodonitrotetrazolium chloride (INT) indicator

The MIC of *T. procumbens* volatile extract was determined by serial dilution, as described by Mogana *et al*.[32] Stock solution of the extract was prepared in 1.5 mL eppendorf tube by dissolving the volatile extract (250 mg/ml) in DMSO. The serial dilutions ranging from 125 mg/mL to 1.95 mg/mL using Mueller–Hinton broth in 96-well microplates were prepared from stock solution. A bacterial suspension containing approximately 5×10^5 colony-forming units/mL was prepared from the 24 h culture plate and this suspension 100 µL was inoculated into each well. The sterile and growth control wells were studied and incubated at 37°C, 24 h for bacteria and 48 h for fungal which required a longer time for growth than the bacteria. After incubation 40 µL of a 0.4 mg/ml solution of p-Iodonitrotetrazolium chloride (INT) was added to each well as an indicator of microbial growth. The plates were incubated again at 37 °C for 30 min for bacteria and 24 hours for fungal. The MIC values were visually determined and the lowest concentration of the volatile extract that displayed no visible growth that is inhibited by the bacterial/fungal growth completely was recorded as the minimum inhibitory concentration and taken as the MIC value. The analysis was repeated twice to confirm the MIC values.

Results and Discussion

The GCMS analysis results of the volatile extracts from *L. sativum* and *T. procumbens* shoots as presented in the Tables 1 and 2 and Figures 1 and 2 revealed a total of nine and twenty compounds, respectively identified in the extracts and their structures are presented in Figure 3. The volatile extract obtained from *L. sativum* shoot consists majorly of fatty acids and its derivatives which include (*Z*)-13-docosenamide, *E*-11-methyl-12-tetradecen-1-ol acetate, petroselinic acid and glyceryl monooleate with reported pharmacological activities not limited to anti-aging, antioxidant, anti-influenza virus, anti-depressant, antimicrobial and anti-inflammatory.[6, 33] Whereas, the volatile extract of the *T. procumbens* shoot contain majorly of sesquiterpenoids such as aromandendrene, caryophyllene, *δ*-cadinene, tau.-cadinol, *β*-eudesmol, xanthoxylin, (*E*)-calamenene, patchoulane and isocaryophyllene that constituted 45% of the total compounds identified in the extract. These compounds are responsible for the characteristic sweet scent of the extract and they are also reported to have pharmacological activities such as antimicrobial, antioxidant, anti-inflammatory, anticancer and many more.[18, 20]

The antioxidant and antidiabetic activities of the volatile extracts evaluated showed some notable inhibitory activities. The DPPH and alpha-amylase inhibitory activities of the volatile extract from *T. procumbens* shoot showed significant inhibitory activities with half-maximal effective concentration EC_{50} values of 47.71 µg/mL and 106.74 µg/mL, respectively when compared to those of *L. sativum* volatile extract as presented in Table 3 with EC_{50} values of 48.63 µg/mL and 134.48 µg/mL, respectively. The EC_{50} values of *T. procumbens* can be compare with the work done by Singh *et al*.[34] which reported similar values for DPPH inhibitory activities of ethanol, methanol and aqueous extracts of *T. procumbens* leaves with IC_{50} values of 72.06, 96.28 and 117.89 µg/mL, respectively. Additionally, in the work of Sonawane *et al*.,[35] the alpha amylase inhibitory activities of petroleum ether, chloroform and methanol extracts of *T. procumbens* plant were evaluated and their IC_{50} values ranging between 10 - 100 µg/mL are similar to those in this study. The significant EC_{50} values recorded for the volatile extract of *T. procumbens* when compared to that of *L. sativum* can be attributed to the classes of phytochemicals found in the volatile extract as presented in Table 2, which are predominantly terpenoids with tremendous pharmacological activities.

Antimicrobial activities of the volatile extracts presented in Table 4 revealed that the volatile extract from *L. sativum* shoot is only susceptible to *Streptococcus pneumonea* with zone of inhibition of 11.5 mm at 100 mg/mL of the extract and all the remaining tested organisms (*Staphylococcus aureus*, *Escherichia coli*, *Aspergillus flavus* and *Aspergillus fumigates*) showed resistance towards the extract. Whereas the volatile extract from *T. procumbens* showed susceptibility towards *Streptococcus pneumonea*, *Staphylococcus aureus* and *Aspergillus flavus* with zone of inhibition of 12, 15.5 and 15.5 mm, respectively at the same concentration (100 mg/mL) but *Escherichia coli* and *Aspergillus fumigates* showed resistance towards the extract.

The values of zone of inhibition from this study can be correlated to the values of 15.3, 16 and 16 mm recorded for *Streptococcus pneumonea*, *Escherichia coli* and *Staphylococcus aureus*, respectively as reported by Manjamalai *et al*.[36] at 50 µg/mL essential oil from the leaves of *T. procumbens.* The minimum inhibitory concentration MIC was obtained only for the volatile extract from the *T. procumbens* through the microbroth dilution method using the p-iodonitrotetrazolium chloride (INT) as indicator. The MIC results for *Staphylococcus aureus*, *Escherichia coli* and *Aspergillus flavus* were recorded as 31.25, 62.5 and 62.5 mg/mL, respectively. *Streptococcus pneumonea* was not subjected to MIC determination and *Aspergillus fumigates* showed resistance towards the extract. The superior antimicrobial activities of the volatile extract from *T. procumbens* over the extract from *L. sativum* can also be related to the classes of phytochemicals found in the extract as mentioned earlier.

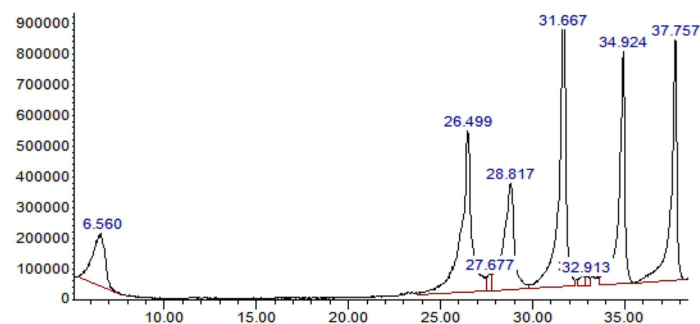

Figure 1: GCMS spectrum of the volatile extract from *Lepidium sativum* shoot

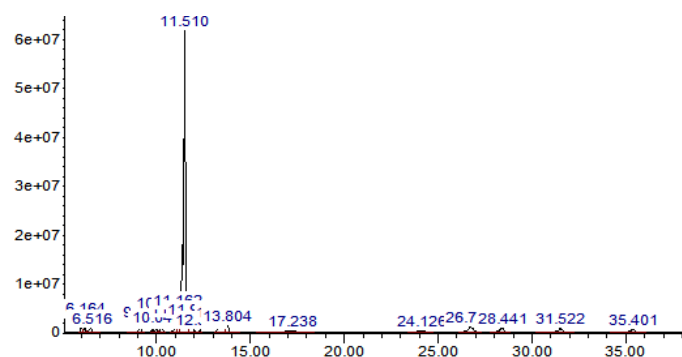

Figure 2: GCMS spectrum of the volatile extract from *Tridax procumbens* shoot

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Table 1: Phytochemicals from volatile extract of *Lepidium sativum* LP shoot

Peak (Compd)	Retention Time	Relative Abundance	Compound Name	Mol. Weight g/mol	Mol. Formula	Compound Class
1	6.5602	7.93	Ethyl E-11-hexadecenoate	282.4614	$C_{18}H_{34}O_2$	Fatty acid ester
2	26.4991	19.86	(Z)-13-Docosenamide	337.5829	$C_{22}H_{43}NO$	Fatty acid amide
3	27.6769	0.66	E-11-Methyl-12-tetradecen-1-ol acetate	268.4350	$C_{17}H_{32}O_2$	Fatty acid ester
4	28.8166	13.85	Petroselinic acid	282.4614	$C_{18}H_{34}O_2$	Fatty acid
5	31.6665	23.89	(Z)-N-(4-fluorophenyl)-5-(methylimino)-2,5-dihydro-1H-1,2,4-triazol-3-amine	207.0920	$C_9H_{10}FN_5$	Amine compound
6	32.7383	0.65	Elaidic acid	282.4614	$C_{18}H_{34}O_2$	Fatty acid
7	32.9131	0.46	Petroselinic acid	282.4614	$C_{18}H_{34}O_2$	Fatty acid
8	34.9243	15.72	Glyceryl monooleate	356.5399	$C_{21}H_{40}O_4$	Monoradyglycerols compound
9	37.7569	16.98	Trimethyl(5-methyl-2-isopropylphenoxy)silane	222.3987	$C_{13}H_{22}OSi$	trimethylsilyl ether compound

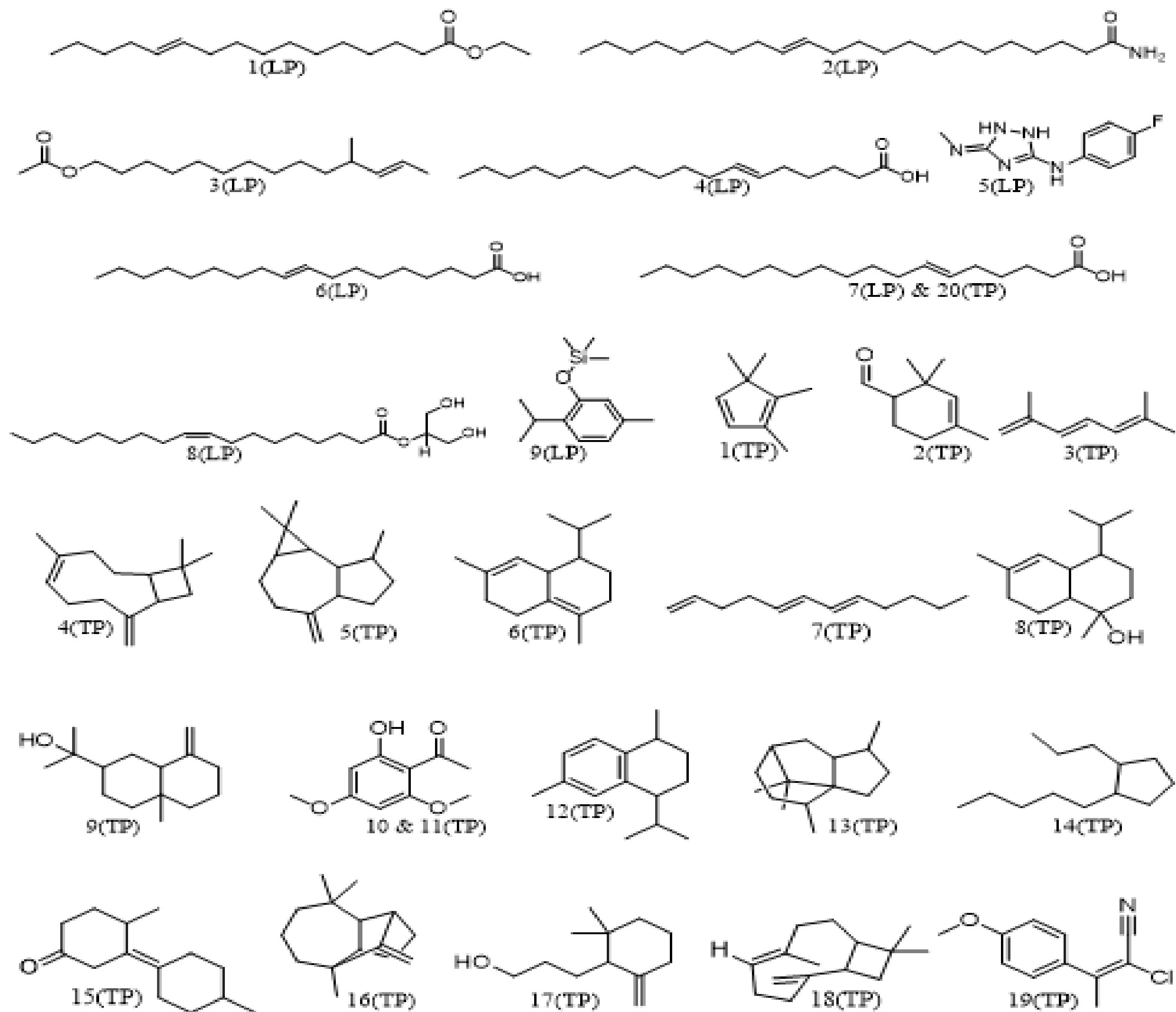

Figure 3: Structures of phytochemicals from volatile extracts of *Lepidium sativum* (LP) and *Tridax procumbens* (TP) shoots

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Conclusion

T. procumbens and L. sativum plants have served as functional food to many in different communities across the Northern region in Nigeria. In this study, the plants have shown to contain phytochemicals with antimicrobial, anti-diabetic and antioxidant activities and these activities supported the ethnomedicinal uses of the plants. However, further studies need to be carried out in area such as *in vivo* analysis, *in silico*, kinetics and mechanisms of actions to explore their wider pharmacological potentials.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 2: Phytochemicals from volatile extract of *Tridax procumbens* TP shoot

Peak (Compd)	Retention Time	Relative Abundance	Compound Name	Mol. Weight g/mol	Mol. Formula	Compound Class
1	5.9852	1.57	1,2,5,5-Tetramethyl-1,3-cyclopentadiene	122.21	C_9H_{14}	Unsaturated hydrocarbons
2	6.1644	1.82	2,2,4-Trimethyl-3-cyclohexene-1-carboxaldehyde (cyclocitral)	152.23	$C_{10}H_{16}O$	Monoterpenoid
3	6.5158	1.83	(E)-2,6-Dimethyl-1,3,5-heptatriene	122.21	C_9H_{14}	Unsaturated hydrocarbons
4	9.1685	2.16	Caryophyllene	204.35	$C_{15}H_{24}$	Sesquiterpenoid
5	9.7613	0.54	Aromandendrene	204.36	$C_{15}H_{24}$	Sesquiterpenoid
6	10.0403	1.02	δ-Cadinene	204.35	$C_{15}H_{24}$	Sesquiterpenoid
7	10.2806	3.51	(5Z,7E)-dodeca-1,5,7-triene	164.29	$C_{12}H_{20}$	Unsaturated hydrocarbons
8	11.0145	3.12	.tau.-Cadinol	222.37	$C_{15}H_{26}O$	Sesquiterpenoid
9	11.1621	4.29	β-Eudesmol	222.37	$C_{15}H_{26}O$	Sesquiterpenoid
10	11.5104	51.99	Xanthoxylin	196.20	$C_{10}H_{12}O_4$	alkyl-phenylketones
11	11.9462	4.09	Xanthoxylin	196.20	$C_{10}H_{12}O_4$	alkyl-phenylketones
12	12.3218	0.50	(E)-calamenene	202.34	$C_{15}H_{22}$	Sesquiterpenoid
13	13.4238	4.66	Patchoulane	206.37	$C_{15}H_{26}$	Sesquiterpenoid
14	13.8036	2.40	1-Pentyl-2-propylcyclopentane	182.35	$C_{13}H_{26}$	Saturated hydrocarbon
15	17.2376	3.15	4',6-dimethyl-1,1'-bi(cyclohexyliden)-3-one	206.32	$C_{14}H_{22}O$	cyclohexylidene cycloketone
16	24.1264	1.77	Longifolene	204.36	$C_{15}H_{24}$	Sesquiterpenoid
17	26.7259	3.77	2,2-dimethyl-6-methylene-cyclohexanepropanol	182.31	$C_{12}H_{22}O$	Alcohol
18	28.4407	2.84	Isocaryophyllene	204.35	$C_{15}H_{24}$	Sesquiterpenoid
19	31.5219	2.75	2-Chloro-3-(4-methoxyphenyl)but-2-enenitrile	207.65	$C_{11}H_{10}ClNO$	Nitrile
20	35.4006	2.24	Petroselinic acid	282.46	$C_{18}H_{34}O_2$	Fatty acid

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Table 3: 2,2-diphenyl-1-picrylhydrazyl DPPH radical and alpha-amylase inhibitory activities of LP and TP volatile extracts

S/No	Extract	Concentration ppm (µg/mL) 150	100	50	20	10	EC_{50} value µg/mL
		% Inhibition					
	DPPH						
1.	LP	33.52 ± 4.60	23.09 ± 4.71	16.23 ± 5.83	9.90 ± 6.41	0.76 ± 4.06	48.63
2.	TP	18.60 ± 4.57	14.89 ± 5.95	11.43 ± 3.55	8.20 ± 2.96	4.73 ± 0.47	47.71
3.	Ascorbic acid	83.06 ± 2.99	73.13 ± 1.54	62.41 ± 2.88	41.77 ± 1.54	31.44 ± 1.78	41.69
	α-amylase	**300**	**200**	**150**	**100**	**50**	
		% Inhibition					
1.	LP	38.12 ± 5.72	32.42 ± 5.98	25.77 ± 4.49	9.62 ± 3.35	3.09 ± 1.30	134.48
2.	TP	24.94 ± 2.88	23.99 ± 1.79	20.19 ± 3.27	9.50 ± 5.57	-2.14 ± 2.88	106.74
3.	Acarbose	63.66 ± 1.23	49.64 ± 4.18	28.50 ±7.42	14.01 ± 2.18	9.50 ± 2.47	165.93

% Inhibition = Mean ± Standard deviation of n = 3

Table 4: Antimicrobial susceptibility (mm) and minimum inhibitory concentration (MIC) mg/mL results of LP and TP volatile extracts

Antimicrobial susceptibility; agar well diffusion result (mm)						
S/No	Extract	***Streptococcus pneumonea***	***Staphylococcus aureus***	***Escherichia coli***	***Aspergillus fumigates***	***Aspergillus flavus***
1.	LP	11.5	R	R	R	R
2.	TP	12	15.5	R	R	15.5
Minimum Inhibitory Concentration (MIC) mg/mL using INT assay						
1.	TP	ND	31.25	62.5	R	62.5

R = Resistant ND = Not Detected

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