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INVESTIGATION OF ANTI-BROWNING ACTIVITY AND ANTIMICROBIAL ACTION OF *TRIDAX PROCUMBENS* LEAF

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ABSTRACT: *Browning of Yams due to Oxidative and Microbial action poses a major problem to food quality, shelf-life, and economic value. The present study investigated the inhibitory effect of ethanolic and n-hexane extracts of Tridax procumbens on Oxidative browning in Yams. The plant material was extracted by Soxhlet extraction, and the concentrate was used to determine anti-browning activity compared with other standards such as Citric, Ascorbic, Hot water, and Cold water using spectrophotometric measurement. Antioxidant activity was determined using DPPH, FRAP, and TPC. Antimicrobial activity was carried out using the disk diffusion method, and the Phytochemical Screening was determined by GC-MS methods. The results of Anti-browning activities of the samples A-ethanolic and B-hexane extracts showed high absorbance of 1.64±1.47^{c} and 1.69±1.54^{c}. The Antimicrobial activity of samples (A and B) revealed a high Zone of Inhibition (ZOI) of 19±2, 15±1, and 17±2 for E. coli, S. aureus, and B. subtilis microorganisms, respectively. These results revealed that Tridax procumbens extracts have anti-browning and antimicrobial activities, which may be traceable to the presence of Naphthalene, 2,6-dimethyl, and 1-Tridecene, which have antimicrobial and antioxidant activities. These findings highlight its potential application in food processing and preservation.*

KEYWORDS: Tridax procumbens, Anti-browning, Anti-microbials, antioxidant, Dioscorea spp.

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INTRODUCTION

Yams, Dioscorea spp., are a crucial source of carbohydrates for millions of people, particularly in sub-Saharan Africa, where it is widely cultivated. In West Africa, yams are an essential staple food, especially for the Tiv people of Benue State, Nigeria. As the second most important root/tuber crop in the region after cassava, yams play a significant role in food security and poverty alleviation, with their cultivation providing numerous economic opportunities (Aidoo et al., 2011). However, despite its agricultural importance, yams face significant post-harvest challenges, with approximately 30% of the annual yield lost due to spoilage (Addy, 2012). These losses are primarily driven by factors such as inadequate storage, diseases, pests, and, notably, enzymatic browning (Anaadumba, 2013).

Enzymatic browning, caused by polyphenol oxidases (PPOs) and peroxidases (PODs), is one of the primary culprits for yam deterioration. Upon cutting or physical damage, the enzymes catalyze the oxidation of phenolic compounds, leading to the formation of brown pigments known as melanins, which negatively affect the nutritional quality and visual appeal of yams (Kaur & Singh, 2009; Xiao et al., 2017). This enzymatic activity is a significant hurdle in yam processing, as it compromises both the sensory and nutritional properties of the tubers, limiting their shelf life and value (Adegoke et al., 2017). These enzymes catalyze the oxidation of phenolic compounds, resulting in the formation of o-quinones that undergo polymerization to produce brown pigments. This process not only alters the organoleptic properties, such as the appearance of the yams, but also results in the loss of essential nutrients and the degradation of sensory qualities.

To combat browning and extend the shelf life of yams, various preservation methods, including chemical preservatives such as ascorbic and citric acids, have been explored. However, concerns about the health implications of commonly used chemical anti-browning agents have led to growing interest in natural alternatives (Okafor et al., 2021).

Tridax procumbens, also known as “coat buttons,” is a perennial plant in the Asteraceae family that originated in Central America but is now found throughout the tropics and subtropics. It is a highly valuable plant with a maximum number of pharmacological activities and is one of the essential ingredients in most of the compound preparations of Ayurvedic literature (Kethamakka & Deogade, 2014). Traditionally, it is used for the treatment of bronchial catarrh, dysentery, malaria, high blood pressure, and to check haemorrhage from cuts, bruises, and wounds, and as a therapy for bleeding backache and diarrhoea. Extracts from the weed are reported to possess anti-diabetic and antibacterial properties and can also act as anti-hepatotoxic and anti-oxidant (Debolina et al., 2022). Current reviews have presented a systematic study of the plant with ethno-pharmacological relevance and scientific evidence citing the importance of the plant to indigenous people as well as pharmaceutical industries, without making mention of its anti-browning activities, which is often undesirable in food processing and preservation. Several studies have indicated that natural compounds with antioxidant and antimicrobial activities may offer promising solutions to anti-browning activities (Babajide et al., 2020; Ukwuani et al., 2022). The bioactive compounds present in *Tridax procumbens* can potentially scavenge free radicals and inhibit the enzymatic activity responsible for oxidative browning, thereby prolonging the shelf life of yams. Furthermore, these extracts possess antimicrobial properties, which could protect the tubers from microbial contamination during storage and transport, addressing another major cause of post-harvest

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losses (Ezekiel et al., 2019). *Tridax procumbens*, known for its rich phytochemical composition, including flavonoids, tannins, alkaloids, and phenolic compounds, has been traditionally used to delay enzymatic browning and protect against microbial spoilage (Ukwuani et al., 2022).

Despite its widespread use in local practices, to the best of our knowledge, limited studies have explored its potential potential for post-harvest yam preservation has not been studied. It has been the subject of research for various medicinal properties (antimicrobial, anti-inflammatory, and antioxidant activities) but there hasn't been any report in literature focusing specifically on the utilization of *Tridax procumbens* to mitigate or prevent oxidative browning, even though preliminary phytochemical investigation has shown the presence of flavonoids, alkaloids and phenolic acids which are known to play significant roles in protecting cells from oxidative damage. Therefore, this study aims to evaluate the anti-browning and antimicrobial activities of Tridax procumbens leaf extracts for potential application in yam preservation.

MATERIAL AND METHODS

Chemicals and reagents

Ethanol, n-hexane, citric acid, ascorbic acid, nutrient agar, and distilled water were all of analytical grade. DPPH FRAP and TPC (Sigma Aldrich USA).

Collection and preparation of plant material and yam samples

The plant materials *Tridax procumbens* leaves were collected from Akperan Orshi Polytechnic during the wet season. The plant was identified and authenticated by a botanist at the forestry department of the polytechnic. Fresh Yam tubers were bought from Gboko market and were selected based on freshness, absence of physical damage, and maturity. The plant material (leaves) was washed thoroughly with distilled water and dried at room temperature until it reached a constant dry weight. The leaves were then pounded into powder and stored in airtight containers. The powdered leaves were subjected to Soxhlet extraction at temperatures above 78^0c and 69^0_c using 50grams of the powder and 500mL for each solvent (ethanol and n-hexane). The extraction was completed after 48 hours, with the solvent being replenished as required to ensure maximum extraction. The solvent was removed by allowing it to air dry at laboratory temperature. The resulting concentrated extracts were used for further analysis. The yam samples were washed thoroughly under running tap water to remove soil, debris, and surface contaminants. They were peeled using a sterile stainless-steel knife to prevent contamination. Furthermore, they were sliced into a uniform thickness of 5mm and pre-treated immediately by immersion in distilled water to prevent early browning before treatment (Olowokere & Falodun, 2020; Okorondu et al., 2021).

Determination of anti-browning activity

Anti-browning activity of the yam slices was done using different preservative treatments compared to control samples (untreated), which were exposed to air to observe the natural rate of oxidative browning. Citric acid was applied at a concentration of 2% (w/v) by submerging the yam slices into the citric acid solution for a period of 5- 10 minutes for observation, and allowed to drain for further analysis. Similarly, ascorbic acid and absolute ethanol were applied

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by dipping yam slices in 1% and 10% solutions, respectively, for 10 minutes, after which they were dried and stored for colour evaluation. For the plant extract, yam slices were also treated with 10% ethanolic and n-hexane extracts, respectively, for 10minutes. Physical methods, such as cold water treatment, were done by dipping the yam slice into cold distilled water for 10 minutes. The blanching of the yam slices in hot water was done at 70-80 °C for 2-3minutes, after which it was cooled in ice water. Each of these treatments was compared against the control (Ali et al., 2016).

Colour measurement and browning assessment.

The extent of oxidative browning on physicochemical treatments compared to the ethanol and n-hexane extract treatments was determined using a UV-Vis spectrophotometer at 420nm wavelength. The yam slices already treated, along with the control for each treatment, were chopped and made into a slurry with distilled water. It was then filtered with Whatman filter paper, and the absorbance for the control and all other treatment groups was taken in triplicate at 420nm respectively. (Ali et al., 2016)

Determination of antioxidative activity

The anti-oxidative effects of ethanolic and n-hexane extracts of *Tridax procumbens* were assessed using three well-established assays (DPPH, FRAP, and TPC) to measure their capacity to scavenge free radicals and reduce oxidative stress.

DPPH (2, 2-diphenyl-1-picrylhydrazyl) Assay

The scavenging ability of extracts against the oxidant (DPPH) was measured using a UV-Vis spectrophotometer at 517nm, and the results were compared to the physicochemical parameters (citric and ascorbic acids, absolute ethanol, cold and hot water) (Chen et al., 2021)

FRAP (Ferric reducing antioxidant power) Assay

The FRAP assay was done by measuring the change in absorbance at 593 nm. A standard curve was prepared using ascorbic acid, and the results were expressed in terms of ascorbic acid concentration equivalence (Chen et al., 2021)

TPC (Total phenolic content) Assay

The TPC of the extracts was determined using the Folin-Ciocalteu method. The results were expressed in terms of milligrams of gallic acid equivalents per gram of dry weight (Adeyemi et al., 2020)

Antimicrobial assay

The antimicrobial activities of the ethanolic and n-hexane extracts of *Tridax procumbens* were assessed against a range of bacterial and fungal pathogens using the disk diffusion method. The selected pathogens included Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungal strains (*Candida albicans*, *Aspergillus niger*). These microorganisms were chosen due to their relevance in food spoilage. For each microorganism, a bacterial or fungal suspension was prepared and spread evenly on the surface of nutrient agar for bacterial strains and Potato

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Dextrose Agar (PDA) for fungal strains. Sterile filter paper discs were impregnated with various concentrations of the plant extracts (10, 50, 100, and 200 µg/mL) and placed on the inoculated agar plates. The plates were incubated at 37°C for 24–48 hours for bacterial cultures and 72 hours for fungal cultures. The zone of inhibition around each disc was measured to determine the antimicrobial activity of the extracts (ethanolic and n-hexane) accordingly. (Adebayo et al., 2018; Olowokere and Falodun, 2020).

Determination of phytochemical composition using GC-MS

Gas chromatography-mass spectrometry was used to determine the phytochemical constituents of the ethanolic and n-hexane extracts. The extracted compounds were volatilized and separated on a capillary GC column based on polarity and boiling point; the mass spectrometer provided molecular ion patterns for structural identification. Spectral data were matched with those in the NIST/Wiley libraries, and retention indices were compared with literature values for confirmation. The relative abundance of each constituent was expressed as a percentage of its peak area in the chromatogram. (Adeniyi et al., 2019)

Statistical analysis

Data was statistically analysed using SPSS, ANOVA version 26 to determine whether significant differences among treatments were necessary. All determinations were done in triplicate, and mean values were compared using Tukey's post hoc test at a significance level of $P < 0.05$

RESULTS AND DISCUSSION

Tables 1: Anti-browning activities of ethanolic and n-hexane *T. procumbens* extract

Parameter	Absorbance	Observation
Control Sample	$0.35^{a}\pm0.032$	White
Citric acid	$0.52^{ba}\pm0.493$	Pale yellow
Ascorbic acid	$0.69^{b}\pm0.641$	Pale red
Cold water	$0.61^{b}\pm0.562$	Light brown
Hot water	$0.66^{b}\pm0.604$	Dull white
Absolute ethanol	$1.08^{c}\pm0.994$	White
Sample A	$1.64^{c}\pm1.473$	Lemon green
Sample B	$1.69^{c}\pm1.545$	Dark green

Means of triplicate determination reported within column with the same superscript are not significantly ($P>0.05$) different.

This study investigated the inhibitory effects of *Tridax procumbens* extracts on oxidative browning on yam slices, alongside their antioxidant and antimicrobial properties. The findings provide valuable insights into the potential of *T. procumbens* as a natural bio-preservative and therapeutic agent.

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Anti-Browning activity of plant extracts

The anti-browning analysis revealed that *T. procumbens* extracts significantly reduced enzymatic browning in yam slices compared to physicochemical conventional inhibitors such as citric and ascorbic acid, absolute ethanol, and cold and hot water, respectively. The absorbance of Sample A and B, along with the physicochemical parameters, were significantly ($P<0.05$) different compared to the control group, with each parameter presenting a unique observation except for absolute ethanol, which had the same observation as the control group despite its higher absorbance of $1.08^{c}\pm0.994$, which was significantly higher than $0.35^{a}\pm0.032$ of the control. This is probably due to the fact absolute ethanol, with its hydroxyl group, can change the electronic structure of the compounds through the formation of hydrogen bonds with monophenols. The result of this interaction is a hydrogen-bonded complex that significantly affects the electron distribution in the monophenolic molecule. This influences the chemical reactivity of the phenol by stabilizing it and altering the absorbance properties in spectroscopic measurement. Furthermore, the ethanol's role in the experiment may be involved in a direct interaction with phenols present in the tissues of the yam slices, and thus leads to self-interaction (dimerization) that influences the increase in absorbance without visible change in color (Bellamy & Pace, 1966)

Sample A (ethanolic extract of *T. procumbens*) and B (n-hexane extract of *T. procumbens*) demonstrated significant anti-browning activity compared to citric acid, ascorbic cold and hot water, which could not stop the browning action as shown in Table 1 with physical observations of pale yellow, pale red, light brown, and dull white. These colours indicated a gradual browning of the yam slice, as indicated by its higher absorbance values and visible reduction in browning. This suggests that bioactive compounds within *T. procumbens* play an essential role in inhibiting polyphenol oxidase (PPO), the key enzyme in browning reactions (Olayemi et al., 2020; Sun et al., 2022). The lemon green and dark green colouration of samples A and B pigmentation observed may also reflect the presence of chlorophyll derivatives along with total phenolic content and lipophilic compounds that mask and cause interference in the oxidative discoloration and may have caused a baseline shift in the spectrometric measurement, leading to an increase in absorbance without any visible browning activity. These results align with prior reports that polyphenolic plant extracts can outperform traditional physicochemical browning inhibitors in tubers and fruits (Zheng et al., 2021).

Table 2: **Antioxidant activity of plant extract (ethanolic and n-hexane extract)**

Extract/Group	DPPH	FRAP	TPC	IC50 Value
Sample A	$68.71^{a}\pm0.51$	$56.38^{b}\pm0.50$	$16.58^{c}\pm12.14$	122
Sample B	$75.56^{a}\pm0.50$	$63.96^{b}\pm0.46$	$20.146^{c}\pm0.33$	140
Ascorbic Acid	$89.54^{a}\pm0.01$	$78.42^{b}\pm0.02$	$51.82^{c}\pm0.10$	150

Means of triplicate determination reported within rows with the same superscript are not significantly ($P>0.05$) different.

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Antioxidant activity of plant extracts

Antioxidant assays were a confirmatory test for the bioactivity of *T. procumbens*. The radical scavenging activity of Sample A (ethanolic extract of *T. procumbens*) and Sample B (n-hexane extract of *T. procumbens*) on DPPH, FRAP, and TPC revealed that Sample A showed a stronger IC_{50} value; this implies that it has a better minimum inhibitory concentration ($IC_{50=122}$) compared to Sample B with $IC_{50=140}$. It is worthy to note that both samples (A and B) had better inhibitory minimum concentration when compared to the standard (Ascorbic acid) used in the experiment. The total phenolic content (TPC) of 20.146, as shown in Table 2, showed the reason why sample B was dark green and had a higher absorbance compared to sample A, which was lemon green in the anti-browning assay.

The potent antioxidant activity highlights the plant's ability to neutralize reactive oxygen species, supporting its ethno-medicinal use against oxidative stress-related conditions such as inflammation and degenerative diseases (Sengupta et al., 2019).

Table 3: **Antimicrobial activity of plant extracts**

SAMPLE A (*ethanolic T. procumbens)*

MICROBE	ZOI	MIC	MBC	MBC/MIC	STATUS
E coli	17±2	250	500	2:1	Bacteriocidal
S.aureus	20±1	500	1000	2:1	Bacteriocidal
B.subtilis	15±1	1000	1000	1:1	Bacteriostatic
P.aeruginosa	11±3	1000	1000	1:1	Bacteriostatic
C.albiens	15±1	1000	1000	1:1	Bacteriostatic
A.niger	8±3	1000	1000	1:1	Bacteriostatic

Table 4: Antimicrobial activity of plant extracts
SAMPLE B (*n-hexane t.procumbens*)

MICRO	ZOI	MIC	MBC	MBC/MIC	STATUS
S.aureus	15±2	250	250	1:1	Bacteriocidal
E. coli	17±1	500	1000	2:1	Bacteriocidal
B.subtilis	14±1	1000	1000	1:1	Bacteriostatic
P.aeruginosa	10±3	1000	1000	1:1	Bacteriostatic

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C.albiens	13±1	1000	1000	1:1	Bacteriostatic
A.niger	9±3	1000	1000	1:1	Bacteriostatic

Key. ZOI-zone of inhibition, MIC-minimum inhibition concentration, MBC-minimum bacteriocidal concentration

Antimicrobial activity of plant extracts

The antimicrobial evaluation showed selective activity of *T. procumbens* extracts against bacterial and fungal strains. Both samples were effective, as they showed bacteriocidal effect against *Escherichia coli* and *Staphylococcus aureus*. This finding is consistent with earlier studies attributing this activity to total phenolic compounds and essential oils present in the plant (Ezeonu et al., 2021). Interestingly, both sample A and B showed bacteriocidal effect against Gram-positive bacteria *(S. aureus)* and Gram-negative bacteria *(E. coli).*

The remaining strains of bacteria in the experiment were bacteriostatic when exposed to ethanolic and n-hexane extracts of *T. procumbens,* as shown in Table 3. These findings suggest that the sample may be less effective against organisms with complex resistance mechanisms, which may include the gram-positive *Bacillus subtilis* and gram-negative *Pseudomonas aeruginosa,* and especially fungal microbes such as *Candida albicans and Aspergillus niger.* Similar findings have been reported where *T. procumbens* displayed broad antibacterial but weaker antifungal activity (Sengupta et al., 2019).

Taken together, the study demonstrates that *T. procumbens* possesses strong antioxidant, browning inhibitory, and selective antimicrobial activities. These findings justify its traditional applications in food preservation and medicine, while also suggesting potential for incorporation into pharmaceutical and nutraceutical formulations.

SAMPLE A (ethanolic extract)

Table 5 GC–MS Identified Compounds from *Tridax procumbens*

S/N	RT (min)	Name of Compound	Peak Area (%)	Biological Activity
1	5.1668	Tetradecane	3.6597	Antimicrobial, insecticidal/repellent, hydrophobic carrier of bioactives.
2	5.3817	Naphthalene, 2,3-dimethyl	0.435	Antimicrobial, insecticidal, and antifungal potential.
3	5.5349	Naphthalene, 2,6-dimethyl	3.3433	Antimicrobial, insecticidal, antioxidant activity.
4	6.441	Pentadecane	1.976	Antimicrobial, antifungal, mosquito larvicidal, and contributes to plant defense.
5	7.1149	Naphthalene, 1,6,7-trimethyl	0.7259	Antimicrobial, antifungal, insecticidal, and insect repellent activity.
6	7.6209	1-Tridecene	0.7726	Antimicrobial, antioxidant, and pheromonal (insect communication) activity.

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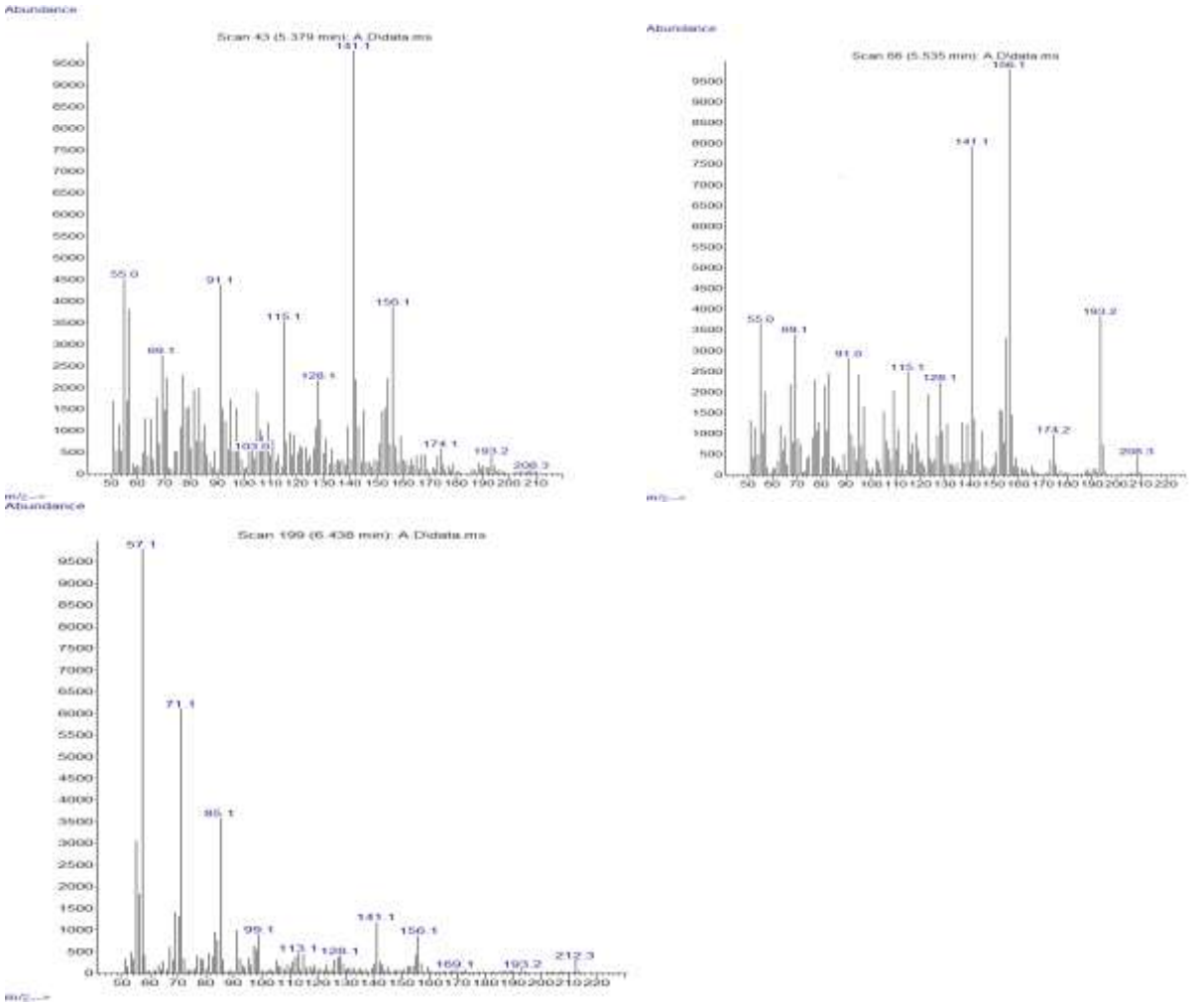
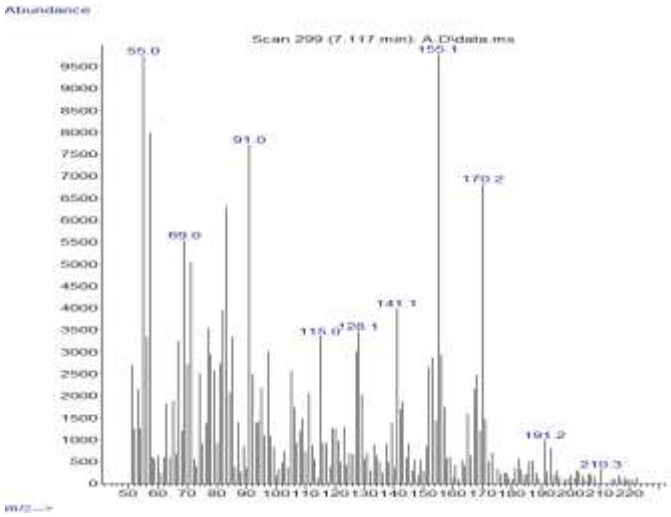
Figures 1, 2, and 3. GC-MS Chromatograms of similar compounds found in Sample A and B of ethanolic and n-hexane extract of *T. procumbens* leaves



Figures 4 and 5. GC-MS Chromatograms of similar compounds found in Sample A and B of ethanolic and n-hexane extract of *T. procumbens* leaves



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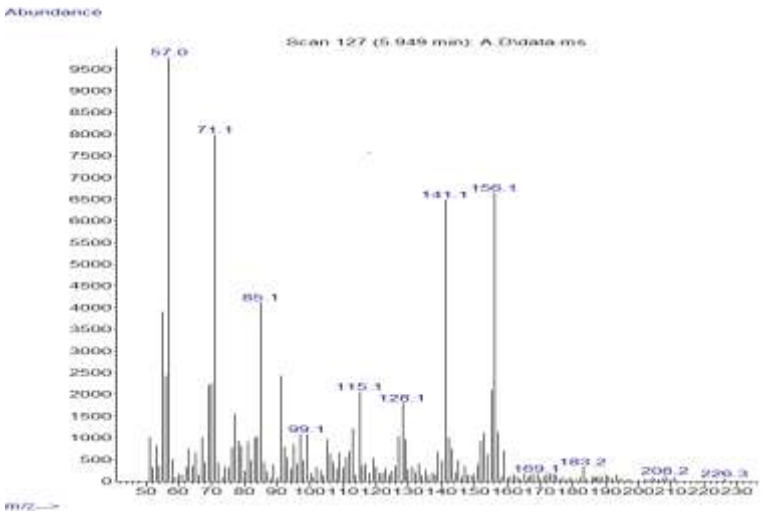


Figure 6. GC-MS Chromatograms of similar compounds found in Sample A and B of ethanolic and n-hexane extract of *T. procumbens* leaves

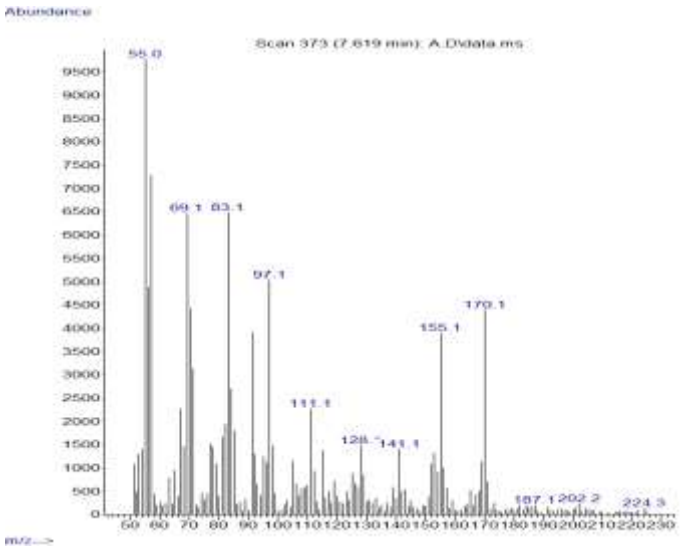
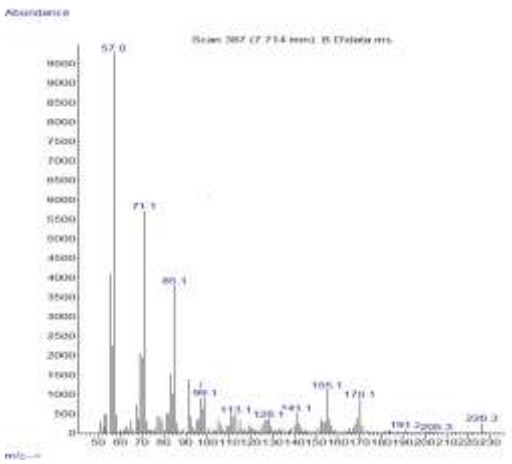


Figure 7.. GC-MS Chromatogram of Sample B of n-hexane extract of *T. procumbens* leaves



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Sample B (hexane extract)

Table 6 GC–MS Identified Compounds from *Tridax procumbens*

S/N	RT	Name of Compound	Peak Area	Biological Activity
1	5.1668	Tetradecane	3.6597	Antimicrobial, insecticidal, allelopathic, mosquito larvicidal activity
2	5.5349	Naphthalene, 2,6-dimethyl	3.3433	Antibacterial, antifungal, insecticidal, insect repellent
3	5.6726	Naphthalene, 2,3-dimethyl	4.1649	Antibacterial, antifungal, insecticidal, cytotoxic properties
4	6.441	Pentadecane	1.976	Antimicrobial, antifungal, mosquito larvicidal, and plant defense
5	7.1149	Naphthalene, 1,6,7-trimethyl	0.7259	Antibacterial, antifungal, insecticidal, insect repellent
6	7.6209	1-Tridecene	0.7726	Antimicrobial, antioxidant, and pheromonal activity
7	7.6949	Undecane	2.8223	Antimicrobial, insect repellent.

Phytochemical screening using GC-MS

One of the most reliable techniques used in identifying the composition of extracts is the GC-MS. The GC-MS analysis of sample A of *T. procumbens* leaf revealed the presence of six compounds (phytochemical constituents) in sample A and seven compounds in sample B, respectively. The peaks in the chromatogram were integrated and compared with the database of spectra of known components stored in the GC-MS library. The chemical constituents identified in sample A (ethanolic extract of *T. procumbens*) are tetradecane, Naphthalene-2,3-dimethyl, Naphthalene-2,6-dimethyl, pentadecane, naphthalene-1,6,7-trimethyl, and 1-tridecene, while Sample B (n-hexane extract of *T. procumbens*) has tetradecane, Naphthalene-2,3-dimethyl, Naphthalene-2,6-dimethyl, pentadecane, naphthalene-1,6,7-trimethyl, and 1-tridecene, and undecane, respectively

GC-MS chromatograms of the peak of the compounds detected in samples A and B are shown in Figures 1, 2, 3, 4, 5, 6, and 7, respectively. The identification of the phytochemical compounds was confirmed based on the peak area, retention time, and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW), and peak area in percentage are presented in Tables 5 and 6. The phytochemicals identified through GC-MS analysis showed many biological activities relevant to this study, as listed in Tables 5 & 6. The biological activities listed are based on Dr. Duke's Phytochemical and Ethno-botanical Databases created by Dr. Jim Duke of the Agricultural Research Service/USDA (Adeniyi et al., 2019)

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CONCLUSION

The present study revealed the essential biological activities of *Tridax procumbens* extracts as a promising natural product for the control of oxidative browning in yam tubers. Furthermore, by outperforming common chemical inhibitors such as citric and ascorbic acid, *T. procumbens* demonstrates added value as a functional bio-preservative. The moderate antioxidant activities, coupled with bactericidal properties exhibited by some of the bacterial strains, highlight their relevance in food preservation and disease prevention. The results further reinforced the therapeutic reputation of *T. procumbens* and support its usage in managing oxidative browning in tubers and control of microbial activities.

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