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ORIGINAL RESEARCH article

Antioxidant, anti-inflammatory, FTIR, and GC-MS analysis of the fractions of *Tapinanthus bangwensis*

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Keywords: Antioxidant, anti-inflammatory, FTIR spectroscopy, GC-MS technique, *Tapinanthus bangwensis*

Abstract: Oxidative stress and inflammation contribute substantially to the various chronic diseases in the world. Many routine drugs are available and in use, but a large proportion of them produce undesirable effects. The study investigated the antioxidant and anti-inflammatory properties and sought to identify the likely compounds present in *Tapinanthus bangwensis*. Antioxidant activity was determined using thiobarbituric acid reactive species (TBARS) and hydrogen peroxide (H_20_2) assays. Bovine Serum Albumin Denaturation (BSAD) and Anti-Proteinase (AP) tests were used to assess anti-inflammatory activity. Identification of the compounds was via gas-chromatography-Mass Spectroscopy (GC-MS), while using FTIR (Fourier transition Infrared) to analyze the functional groups. The TBARs result showed that the reference drug (ascorbic acid), ad HECF 1, exhibited a more substantial inhibition compared to HECF 2. Likewise, the reference drug showed a more substantial inhibitory activity on H_20_2 compared to the fractions. But the inhibition level of HECF 1 increased compared to HECF 2. Aspirin demonstrated a more substantial anti-inflammatory effect compared to the fractions. But that of the HECF 2 was higher compared to the HECF 1. The FTIR result showed the presence of a Carbonyl group (C=O), hydroxyl (OH), and amine group (N-H), suggesting that phenols, carboxylic acids, and fatty acids were likely present. The GC-MS result showed four compounds with reports of antioxidant and anti-inflammatory activity: 2-Pentadecanone, 6, 10, 14-trimethyl, Octacosane, 2, 4-di-tert butyl phenol, and Phytol. Thus, these compounds may contribute to the traditional use of *T. bangwensis* in treating oxidative and inflammatory diseases.

Introduction

Under a long-lasting physiological condition, the human system overproduces oxidant substances (reactive oxygen species) and inflammatory markers that aid in the development of oxidative stress and chronic inflammatory diseases [1]. Specifically, inflammation is a complex biological response (or adaptive process) that organism uses to protect itself or restore homeostasis against the effects of oxidant species, or inflammatory substances, which are produced either through specific pathways, or from certain agents (infections, chemicals, physical, and metabolites). If these inflammatory substances are largely produced, and subsequent stimulation of signal-transduction pathways, it results in mild or chronic inflammatory conditions [2, 3]. This underscores the use of anti-inflammatory drugs in treating cases of inflammation. The core aim of these drugs is to restore homeostasis and protect the patients by simply inactivating the inflammatory processes. Anti-inflammatory drugs are broadly classified into two categories, namely: NSAIDs (non-steroidal anti-inflammatory drugs) and

glucocorticoids. The glucocorticoid inhibits the synthesis of prostaglandins and proteins (or corticosteroids). Conversely, the NSAID inactivates the activity of cyclooxygenases (COXs) - an enzyme that transforms arachidonic acid to thromboxanes, prostaglandins, as well as prostacyclin (inflammatory mediators), which promotes pain, pyretic, and tissue damage [4, 5]. Currently, most inflammatory diseases are well managed using NSAIDs compared to glucocorticoids. This is because NSAIDs exhibit a broader spectrum of activity than glucocorticoids. Some NSAIDs act as COX-1 and COX-2 inhibitors, such as diclofenac, ibuprofen, indomethacin, aspirin, naproxen, piroxicam, sulindac, and oxaprozin, among others. Other act preferentially as COX-2 inhibitors (etodolac, meloxicam, and nimesulide), and those that target COX-2 only (celecoxib). However, these anti-inflammatory drugs relate more substantially to COX-1 inhibition, due to their role in cell sanitization. Although these drugs have shown effectiveness in treating inflammatory diseases, they produce undesirable toxic effects like gastrointestinal, cardiovascular, renal, and hematological effects [6, 3]. This underscores the need for newer anti-inflammatory drugs with good therapeutic qualities (safety, potency, and less toxicity). In line with this, more clinical research efforts should be channeled towards ethnobotanical medicine, and its findings incorporated into modern medicines in drug discovery and development. Reports suggest that plant species harbor varieties and complexity of plant metabolites (natural compounds), that defines their several unique biological activities, including anti-inflammatory and antioxidant activity. However, the major challenge here centers on how to identify these natural metabolites and authenticate their biological activity. To resolve this research gap, there has been a deliberate improvement in the design and development of spectroscopy techniques like HPLC-MS, NMR-MS, LC-MS/MS, and GC-MS/MS, which have assisted in uncovering novel natural compounds in extracts for drug discovery [7]. Presently, ethnobotanical studies cut across several plant species, including African Mistletoe (*Tapinanthus bangwensis*). The plant is a member of the Loranthaceae family, and is prevalent in tropical and subtropical areas of Africa. Though the plant is a parasite, it synthesizes its own food (carbohydrate) via a specific pathway called photophosphorylation. In local languages, it is referred to as *Afomo onisana*, *Awurusie,* and *Kauchi* [8].

Materials and methods

Plant identification and extraction: The procedure of [8] was used. As a reminder, the plant's taxonomical record (*Tapinanthus bangwensis;* Reference number: LUH 4532) had already been filed in the University herbarium for traceability. It started by rinsing the leaves and air-drying at ± 28 °C for five days. They were blended into fine powdered form (200 g), soaked in hexane solvent (2.0 L), and left for 48 hrs. This was followed by filtration, and the liquid recovered was concentrated to form a solid hexane extract (10.31 g). On a chromatographic glass, which has been packed with a solution of silica gel, the extract was then loaded onto it. Adjusting the flow rate to 5mL/min, the content was eluted using different solvents' concentration gradients (hexane and ethyl acetate). The fractions obtained were then resolved into three fractions by considering the same retention factors.

Assessment of the antioxidant activity

Using TBARS Test (Thiobarbituric acid reactive species): The method, as used by Ale E [9], was followed. This began with the rat's liver being excised, washed, refrigerated, and crushed into a fine solution in Tris-HCl buffer (5.0 mM). This was followed by centrifugation at 4000 revolutions per minute for 10 min. The supernatant (100 µL) was withdrawn, and incubated at 37 °C for one hour with varying concentrations of the extracts (20 - 100 µg/mL) in a reaction mixture of 5.0 mM Tris-HCl buffer, and 10 µM ferrous sulphate. The peroxidation process started by introducing 200 µL sodium dodecyl sulfate (8.1%), acetate buffer (500 µL), as well as 500 µL

thiobarbituric acid (0.8%). At a temperature of 100 °C, the solution was incubated for 30 min, and the optical density was measured at 532 nm. The % TBARS inhibition was determined.

Using hydrogen peroxide assay: The % inhibition of H_20_2 was estimated by the previous method [10]. Briefly, a H_20_2 (2.0 mM) solution was prepared in a phosphate buffer at pH = 7.4 (50 mM). Preparing varying concentrations of the extracts (20 - 100 µg/mL), an aliquot (0.1 mL) was transferred into tubes. Immediately, 0.4 mL of 50 mM phosphate buffer was added, followed by the introduction of 0.6 mL of hydrogen peroxide. The tube was then vortexed, and absorbance was taken at 230 nm. The % inhibition of H_20_2 was determined, while using phosphate buffer only (negative control), and ascorbic acid (positive control).

Assessment of the anti-inflammatory activity

Using BSAD test (bovine serum albumin denaturation): The % inhibition level of the extracts was estimated according to the method of [11]. Briefly, the process started by adding the extract in different concentrations (20-100 µg/mL) into tubes, then 1.0% bovine serum albumin solution (0.45 mL) was added. A small amount of hydrochloric acid (1.0 N) was introduced to maintain a pH of 6.3. After this step, it was incubated at 28 °C for 20 min, and then continued heating in a water bath at 55 °C for 30 min. It was allowed to calm, and the absorbance reading was obtained at 660 nm. Dimethyl sulfoxide solution represents the negative control. The % protein denaturation was then determined.

Using an anti-proteinase inhibition assay: The method used by [12] was employed. 1.0 mL of an aliquot of the different extract concentration (20 - 100 µg/mL) was introduced into a tube having 25 mM Tris-HCl buffer (1.0 mL, pH 7.4) and 250 μL of trypsin. The solution was kept in an incubator for five minutes at 37 °C. 1.0 mL of casein solution (0.8% (w/v) was pipetted into the above, and then returned to the incubator for another 20 min. The entire reaction was then stopped following the addition of 2.0 mL of perchloric acid solution (70% v/v), while later centrifuging the cloudy suspension. The hydrolyzed protein (supernatant) was extracted, and at 280 nm, the absorbance was read against the buffer (control). The % proteinase inhibition was determined.

FTIR analysis of Tapinanthus bangwensis: The procedure described previously was employed [13]. The procedure would start with the formation of a thin, transparent disc by mixing the extract with potassium bromide powder. These discs were then placed in the Fourier Transform Infrared Spectrometer (FTIR), which then scanned them to identify the different Phytoconstituents present, over a wide range of wavelengths (600 to 4000).

Spectroscopy analysis of Tapinanthus bangwensis using GC-MS (HECF 2): The gas chromatography (GC) used for this study consisted of a helium carrier gas (flow rate of 0.7 mL/min), column oven temperature set at 70 °C, 5 min in 180 °C, 180 - 260 °C at 3 °C/min, 5 min in 60 °C, 260-280 °C at 0.2 C/min, and 5 min in 280 °C); injector temperature (280 °C), and detector temperature at 290 °C. The condition of the mass spectroscopy (MS) was as follows: Ionization potential (70 eV), ion source temperature (200 °C), and 3000 volts' electron voltage. A small amount of the filtrate was injected into the GC-MS device. The separation of the compounds was carried out under a programmed oven temperature, before reaching an optimized temperature. As the compounds elute, they are detected by the mass spectroscopy, which ionizes the molecules and measures their mass-charge ratio. The compounds were identified by comparing their mass spectral and retention values with those in the Nigerian Institute of Science and Technology database [14].

Statistical analysis: Triplicate data were obtained and transformed into Mean ± S.E.M. The analysis was done on SPPSS software, version 23.0, with a confidence limit at $p < 0.05$. The inhibition concentration (IC_{50}) was estimated via the mathematical expression from the graph.

Results

Antioxidant activity of the fractions on thiobarbituric acid reactive species: **Figure 1** shows that the % inhibition level of ascorbic acid ranges from 17.54 to 72.29%. The % inhibition level of HECF 1 lies between 21.97 to 60.82%. Although ascorbic acid and HECF 1 have a common % inhibition level at 58.0%, there exists a concentration-dependent inhibition level between them. But the % inhibition level of HECF 2 (5.90% to 56.23%) was lower compared to the others. According to **Figure 3**, ascorbic acid (IC_{50} = 4.12 ± 0.01 µg/mL) and HECF 1 (IC_{50} = 4.40 ± 0.00 µg/mL) showed a more significant inhibition level compared to HECF 2 (IC_{50} = 5.21 ± 0.02 µg/mL).

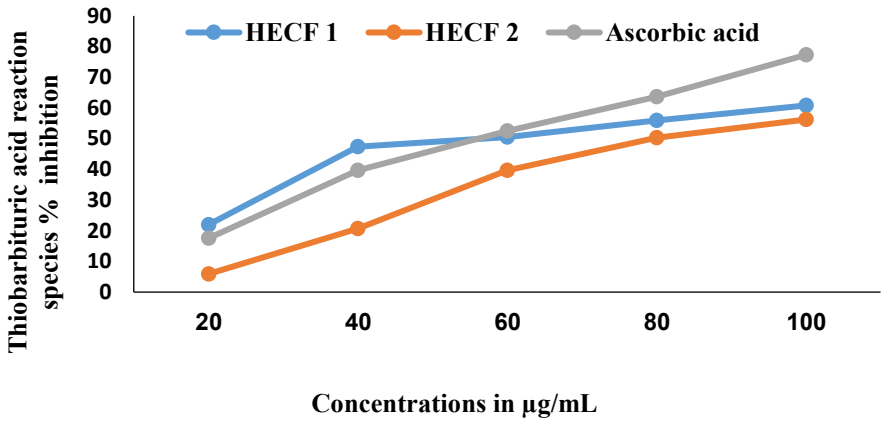

Figure 1: Bioactivity of the fractions of *T. bangwensis* on TBARS radicals

Antioxidant activity of the fractions on H_2O_2: **Figure 2** showed that the % inhibition of ascorbic acid ranged from 24.93 to 63.03%, which was better than that of the fractions. The % inhibition of HECF 1 ranged from 7.22 to 59.73%, while that of HECF 2 ranged from 8.11 to 55.93%. However, both showed a concentration-dependent gradient pattern. Regardless, HECF 1 and HECF showed a common % inhibition level at 26.0% and 50.0%, respectively. In **Figure 1**, ascorbic acid showed a more significant inhibition level (IC_{50} = 3.85 ± 0.00 µg/mL) compared to the fractions. However, the inhibition level of HECF 1 (IC_{50} = 5.26 ± 0.02 µg/mL) was higher compared to HECF 2 (IC_{50} = 5.31 ± 0.01 µg/mL).

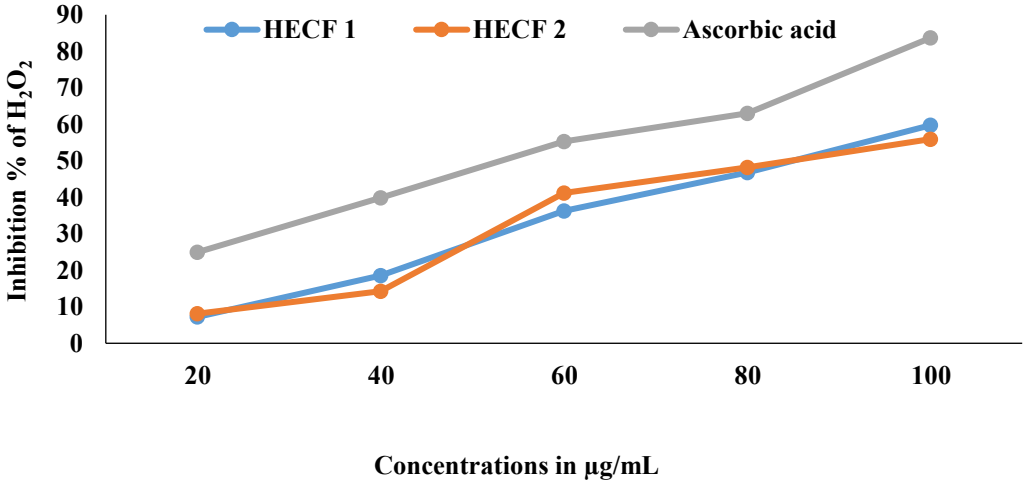

Figure 2: Bioactivity of the fractions of *T. bangwensis* on hydrogen peroxide radicals

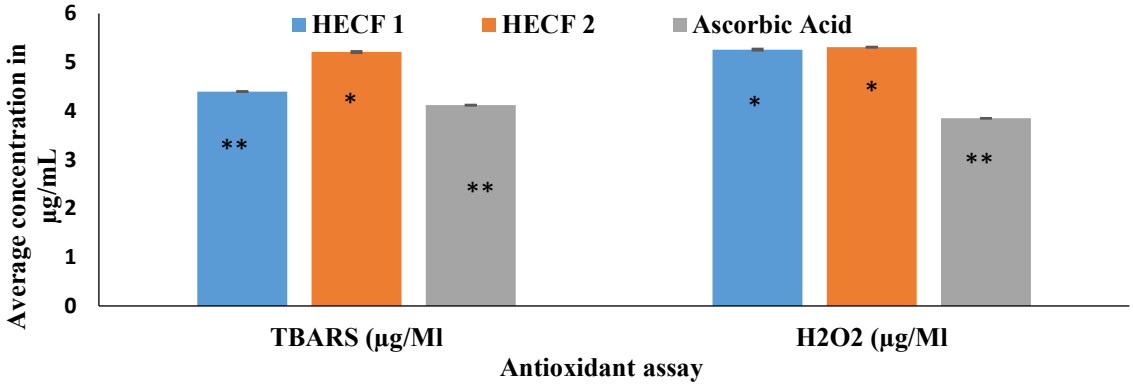

Figure 3: Average concentration (IC_{50}) of the fractions of *Tapinanthus bangwensis*

Anti-inflammatory activity of the fractions using the BSAD assay: **Figure 4** showed that the % inhibition level of HECF 2 (14.35 - 50.99%) was higher compared to HECF 1 (7.49 - 46.75%). However, aspirin (21.84% to 61.01%) showed a better % inhibition level compared to the fractions. As illustrated in **Figure 5**, aspirin showed a significant inhibition level (IC_{50} = 4.57 ± 0.01 µg/mL) compared to the fractions. But, the inhibitory effect of HECF 2 (IC_{50} = 5.61 ± 0.08 µg/mL) was higher compared to HECF 1 (IC_{50} = 6.12 ± 0.01 µg/mL).

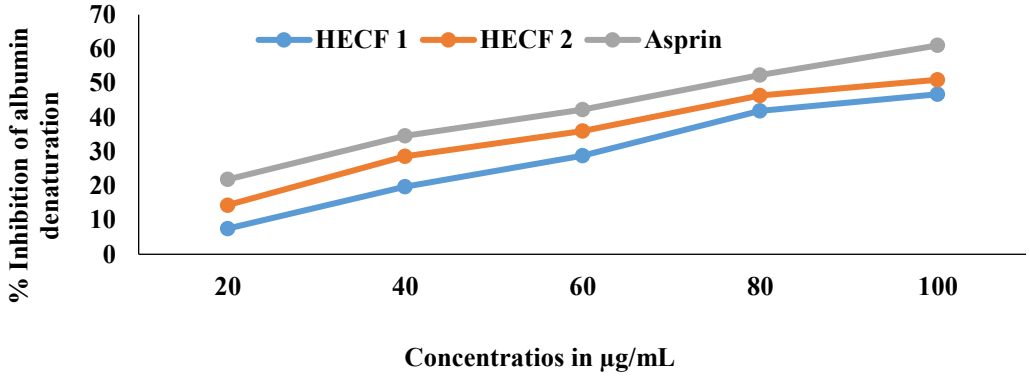

Figure 4: Anti-inflammatory activity of the fractions of *T. bangwensis* using the BSAD assay

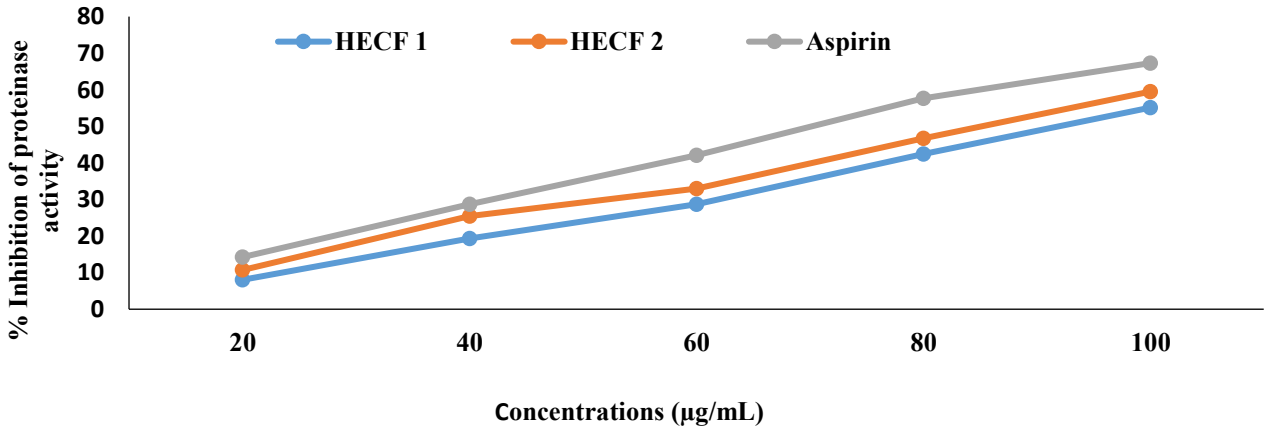

Figure 5: Anti-inflammatory activity of the fractions of *T. bangwensis* using the proteinase assay

Anti-inflammatory activity of the fractions using the Anti-proteinase assay: The result showed that the % inhibition level of the reference drug (Aspirin) was between 14.24 - 67.28%. Moreover, HECF 2 exhibited a % inhibition level between 10.71 - 59.49%, while that of HECF 1 lies within the range of 8.03 to 55.11% (**Figure 5**). As shown in **Figure 6**, the inhibitory effect increased in HECF 2 (IC_{50} = 5.26 ± 0.02 µg/mL) compared to HECF 1 (IC_{50} = 5.70 ± 0.01 µg/mL). But, aspirin (IC_{50} = 4.59 ± 0.01 µg/mL) showed a more substantial inhibition level compared to the fractions.

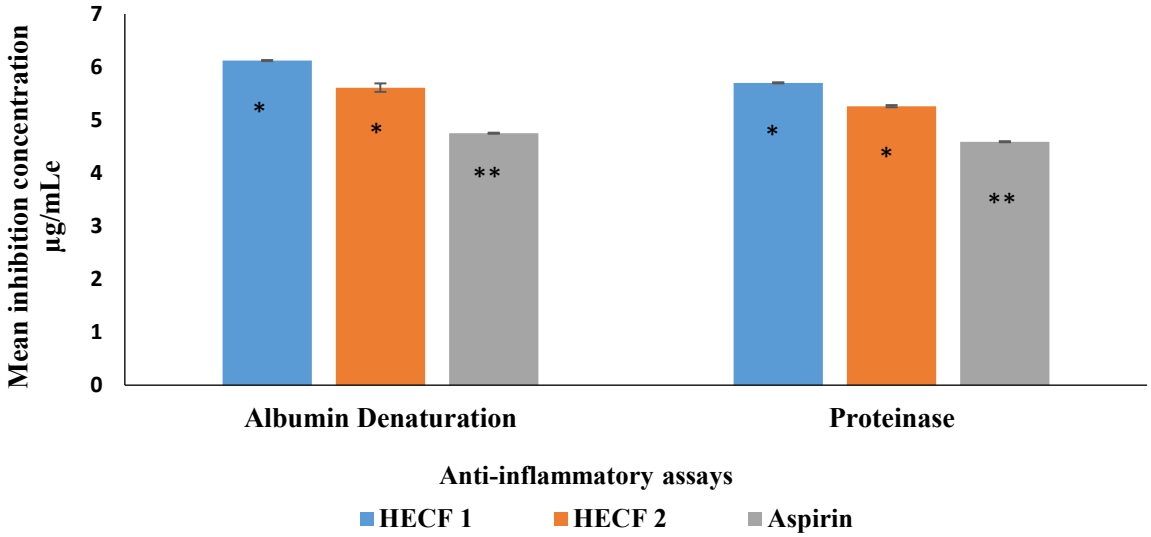

Figure 6: Anti-inflammatory activity of the fractions and aspirin based on the mean inhibition concentrations

FTIR analysis of the fraction of HECF 2: **Table 1** and **Figure 7** showed the different functional groups and the FTIR spectrum, respectively. The FTIR spectrum comprised eleven peaks, with distinct absorption bands. It showed characteristic absorption bands at 721.24 cm^{-1} (alcohol (OH), out-of-plane bend), 883.38 cm^{-1} (peroxides, C-O-O- stretch), and 969.11 cm^{-1} (trans-C-H out-of-plane bend). There were also absorption bands at 1172.25 cm^{-1} (secondary amine, CN stretch), 1377.25 cm^{-1} (aliphatic nitro compounds or nitrate ion), and 1459.25 cm^{-1} (carbonate ion, aromatic ring stretch). On the other hand, it showed absorption bands at 1708.98 cm^{-1} (ketone, carboxylic acid), 1733.21 cm^{-1} (aldehyde, or ester), as well as two bands indicating methylene C-H asym./sym. Stretch (2853.28 cm^{-1} and 2922.23 cm^{-1}), and methyl C-H asym./sym. stretch (2953.92 cm^{-1}).

Table 1: FTIR stereo-isomeric analysis of the fraction of *Tapinanthus bangwensis* (HECF 2)

Peaks	Wavenumbers (cm^{-1})	Intensity	Interpretation
1	721.24	87.32	Alcohol, OH out-of-plane bend
2	883.38	88.49	Peroxides, C-O-O- stretch
3	969.11	82.43	Trans-C-H out-of-plane bend
4	1172.25	81.59	Secondary amine, CN stretch
5	1377.25	78.79	Aliphatic nitro compounds or nitrate ion
6	1459.25	74.76	Carbonate ion, aromatic ring stretch
7	1708.98	76.44	Ketone, carboxylic acid
8	1733.21	79.08	Aldehyde, Ester
9	2853.28	68.93	Methylene C-H asym./sym. stretch
10	2922.23	55.97	Methylene C-H asym./sym. stretch
11	2953.92	69.59	Methyl C-H asym./sym. stretch

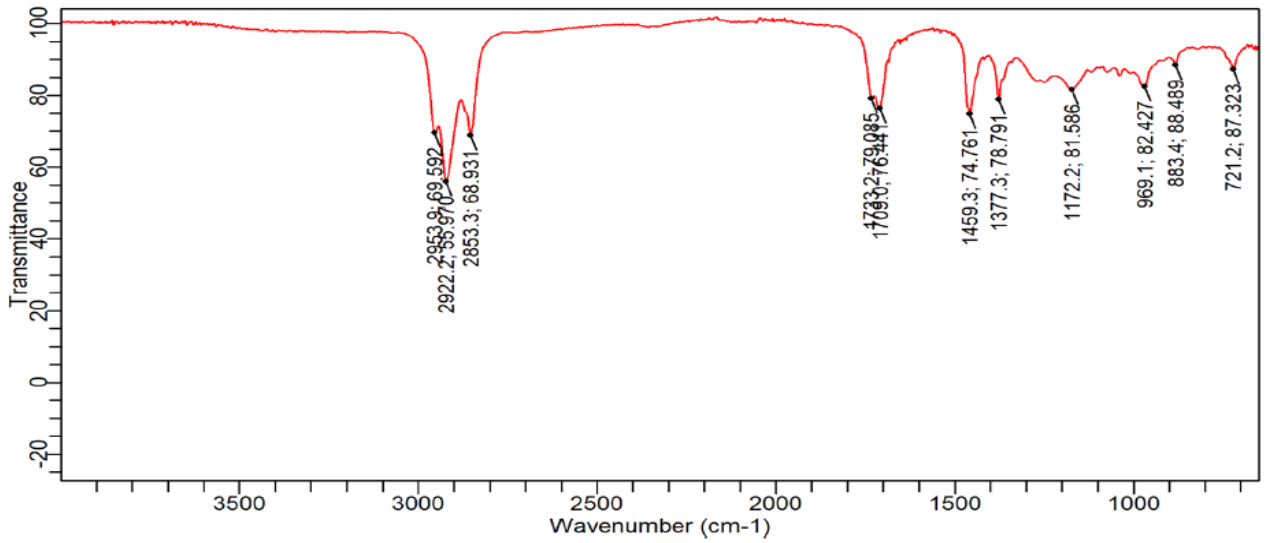

Figure 7: FTIR spectrum of the fraction of *Tapinanthus bangwensis* (HECF 2)

GC-MS phytochemical profile of HECF 2: **Table 2** shows some of the GC-MS-identified compounds, and **Figures 8** and **9** represent the GC-MS chromatogram. The compounds were found to be in the class of phenolic, terpenoids, ketone, alkane, and fatty acids. The result points that 4, 8, 12, 16- tetramethylheptadecan-4-olide (0. 69%), and n-hexadecanoic acid (33.56%) constitute the compounds with the lowest and highest % areas. Also, a benzothiazole derivative compound known as N-(3-Allyl-2-oxo-2, 3-dihydro-1, 3-benzothiazol-6-yl) acetamide (TBDMS derivative) with a % area of 0.75 was identified. The result showed the presence of 3,5-ditert-butyl-4-hydrophenylpropionic acid, and 2,4-Di-tert-butylphenol (phenolic compounds) having % areas as 0.73% and 1.19% respectively. Also present were 9-octadecenoic acid (13.29%), octadecanoic acid (8.13%), and phytol (1.35%). In addition, three alkane compounds were identified, namely, hexadecane (% area = 2.33%), eicosane (% area = 2.88%), and octacosane (% area = 0.77%). A ketone-like compound named 2-pentadecanone, 6, 10, 14-trimethyl, with a % area of 2.29, was also identified.

Table 2: Showing the GC-MS-identified phytocompounds of *T. bangwensis* (HECF 2) and their bioactivities

Retention time (min)	Area in %	Identified compounds	Class of compound	Molecular weight	Molecular formula	Bioactivity	**Refs.**
9.616	0.73	2,4-di-tert-butylphenol	Phenolic	206.32	$C_{14}H_{22}O$	Antioxidant, Cytotoxicity, Anti-inflammatory, and Antibacterial activity.	**22, 23, 30, 31**
9.885	2.33	hexadecane	Alkane	226.45	$C_{16}H_{34}$	Antimicrobial, antioxidant activity	**32**
12.809	2.29	2-pentadecanone, 6,10,14 -trimethyl	Ketones	268.48	$C_{18}H_{36}O$	Hypocholesterolemic, anti-inflammatory, antioxidant, antibacterial activity	**28**
13.381	2.88	eicosane	alkane	282.55	$C_{20}H_{42}$	Anti-inflammatory, analgesic, and antipyretic effects	**25**
13.610	0.75	N-(3-Allyl-2-oxo-2,3-dihydro-1,3-benzothiazol-6-yl) acetamide (TBDMS derivative)	benzothiazole derivative (heterocyclic compound)	248.30	$C_{12}H_{12}N_2O_2S$	Not found	**Not found**
14.131	33.56	N-hexadecanoic acid	fatty acids	256.42.	$C_{16}H_{32}O_2$	Hypocholesterolemic, Anti-androgenic, and Antioxidant activity	**20**

14.222	1.`19	3,5-ditert-butyl-4-hydrophenylpropionic acid	phenolic	278.39.	$C_{17}H_{26}O_3$	Not found	**Not found**
14.463	0.77	octacosane	alkane	394.76	$C_{28}H_{58}$	Anti-inflammatory, antioxidant and antimicrobial activity	**27**
15.001	0.81	phytol	diterpene alcohol	296.53	$C_{20}H_{40}$ 0	Antimicrobial, cytotoxic, anxiolytic, anticonvulsant, antioxidant, anti-nociceptive, and anti-inflammatory activity.	**29** .
15.418	13.29	9-octadecenoic acid, (E)-	fatty acid	282.47	$C_{18}H_{34}O_2$	Anti-inflammatory, Anti-androgenic Anticancer, Hypocholesterolemic and 5-Alpha reductase inhibitor.	**21**
17.135	0.69	4,8,12,16-tetramethyl heptadecan-4-olide	diterpenoid	324.54	$C_{21}H_{40}O_2$	Antioxidant and anticancer activity	**33**

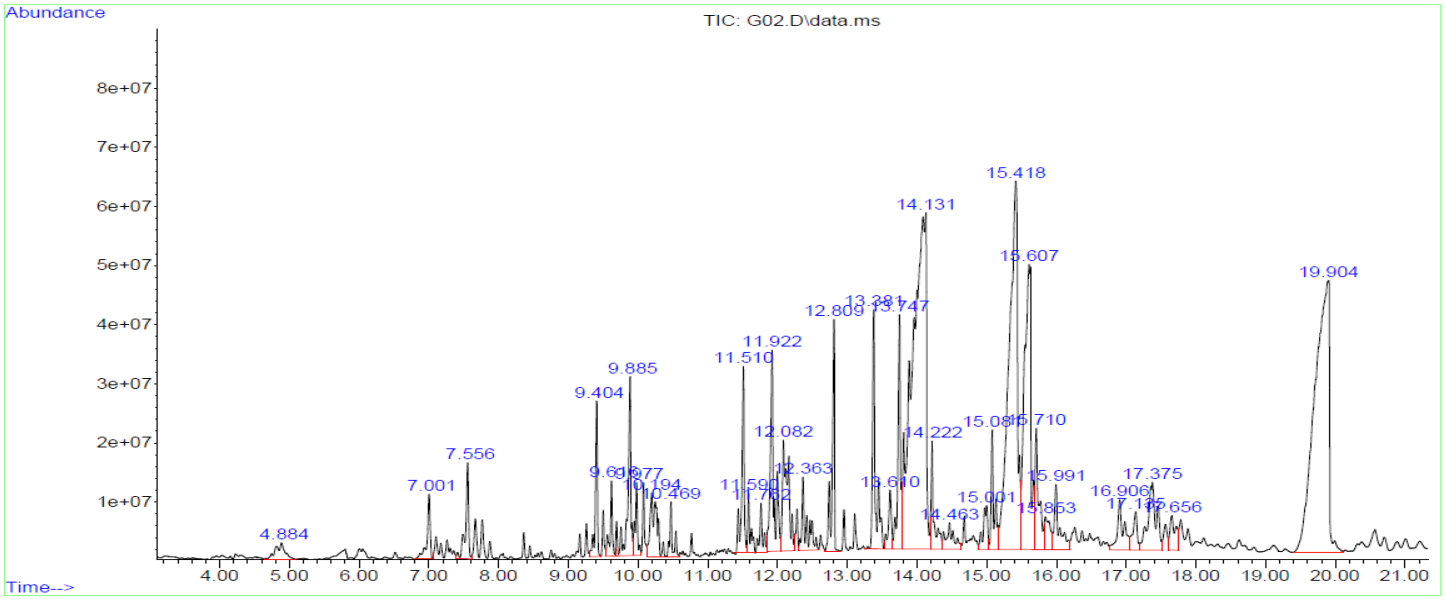

Figure 8: GC-MS spectrum of the HECF 2 fraction of *Tapinanthus. bangwensis*

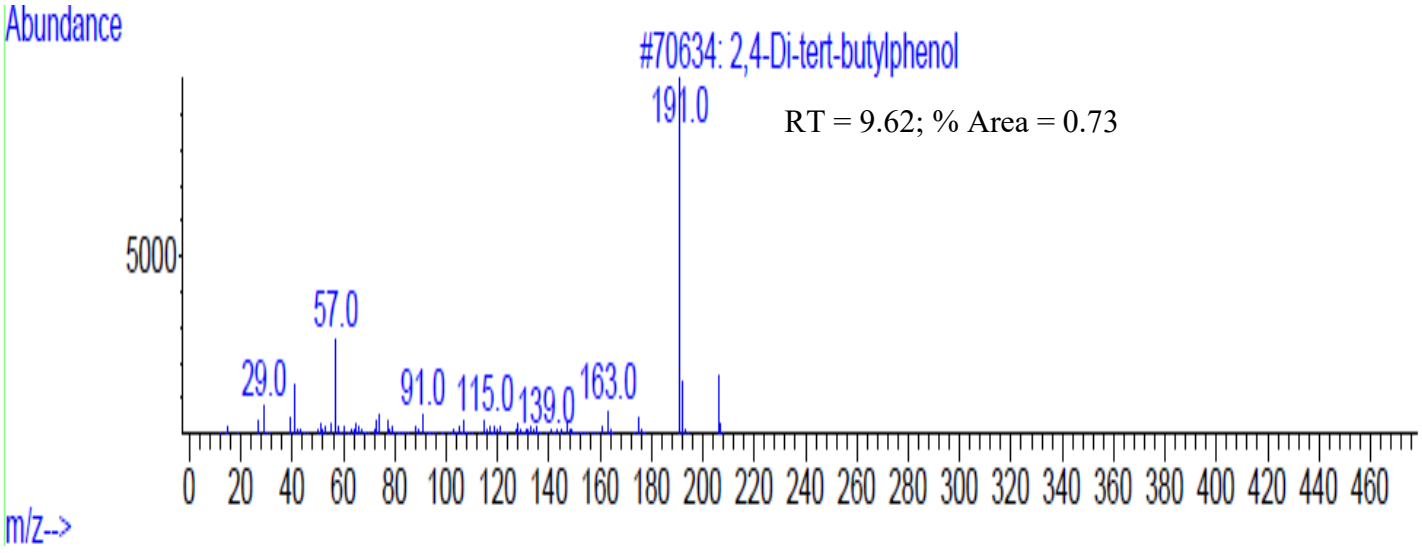

Figure 9A: GC-MS fragments with reported antioxidant and anti-inflammatory activity

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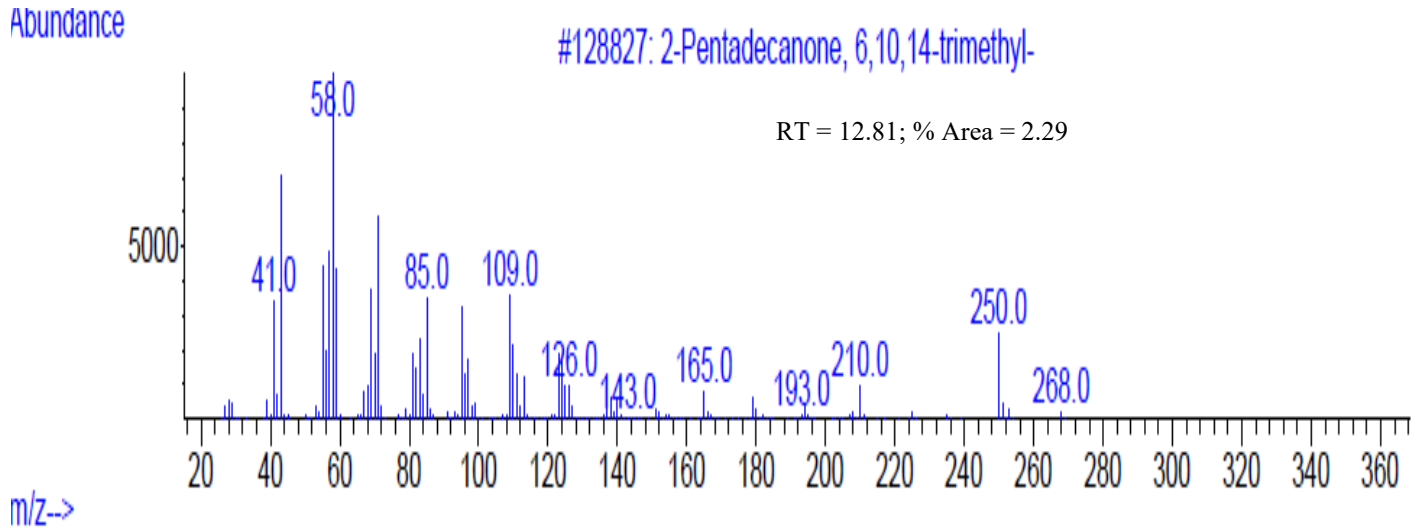

Figure 9B: GC-MS fragments with reported antioxidant and anti-inflammatory activity

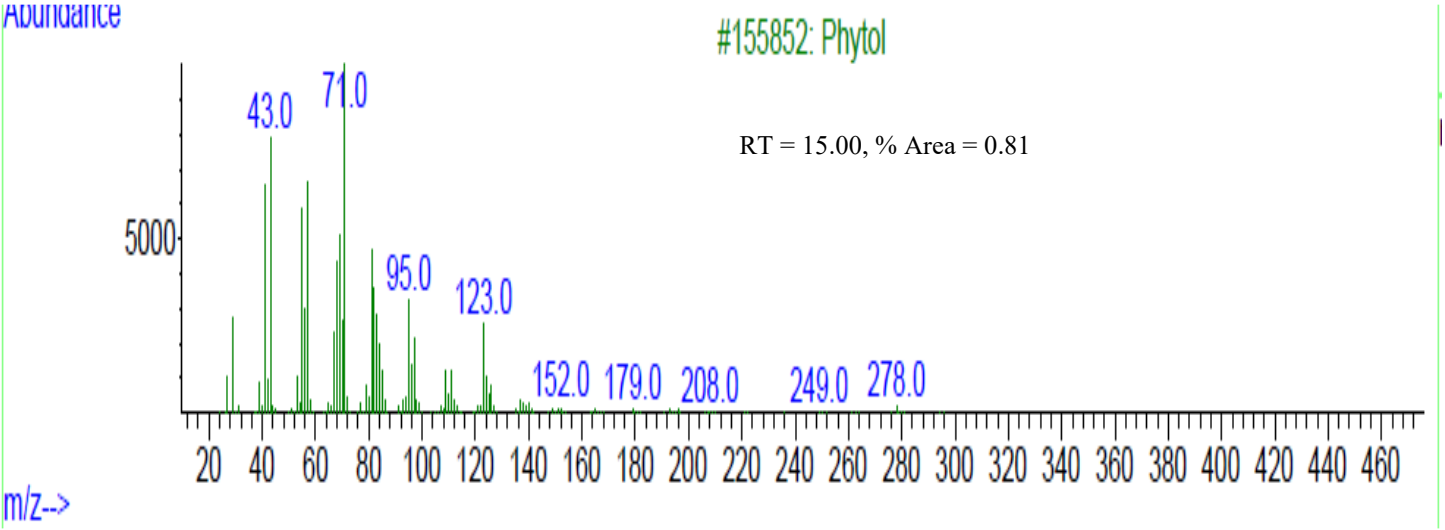

Figure 9C: GC-MS fragments with reported antioxidant and anti-inflammatory activity

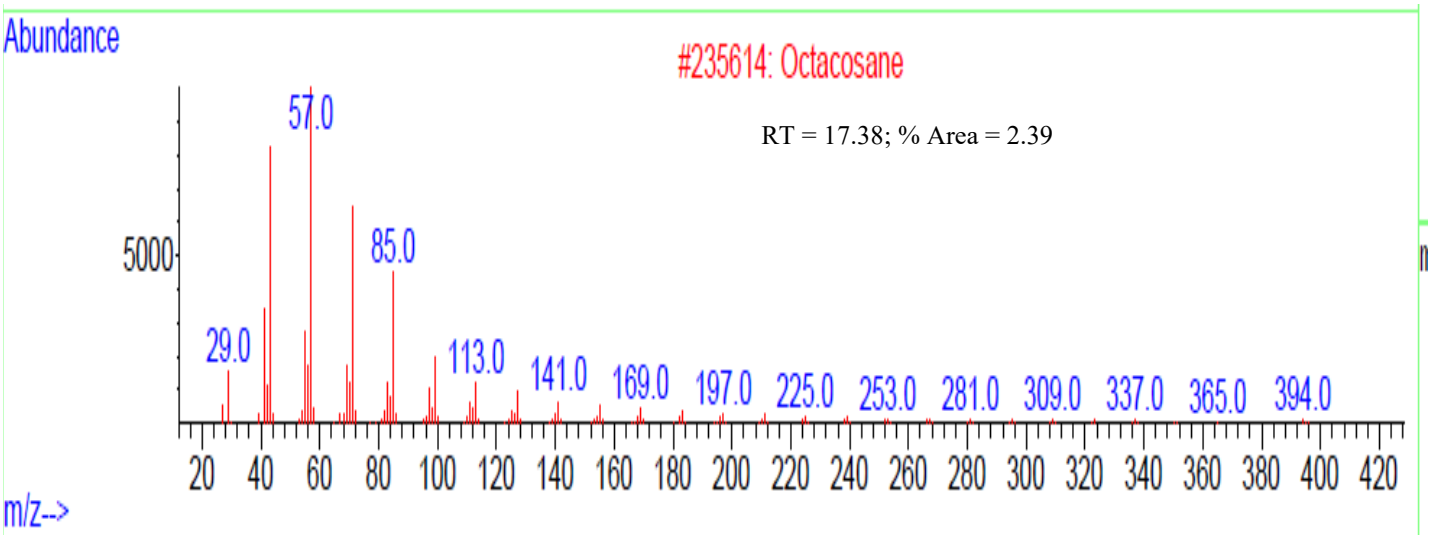

Figure 9D: GC-MS fragments with reported antioxidant and anti-inflammatory activity

Discussion

The current study evaluated the antioxidant, anti-inflammatory, and possible compounds in the *Tapinanthus bangwensis*. The present findings correlated with the report of Ogunbameru and others [1], suggesting that the plant exhibits antioxidant and anti-inflammatory activity. The result of the FTIR spectroscopy showed that the absorption band (721.24 cm^{-1}), corresponding to out-of-plane bending vibration of hydroxyl groups (-OH), typically found in alcohols, suggests the presence of alcohol-based compounds like polyphenols, that demonstrates anti-inflammatory and antioxidant activity [15]. The absorption band at 883.38 cm^{-1} (Peroxide or oxygen-containing compounds C-O-O stretching), and 969.11 cm^{-1} (trans-C-H out-of-plane bending), indicates fatty acids, and contributes to its anti-inflammatory activity [16]. A significant peak at 1172.21 cm^{-1} represents C-N stretching, which may be found in alkaloids, which have diverse pharmacological activities [17]. The peak at 1377.25 cm^{-1} indicates the possible presence of nitrogen-containing compounds like alkaloids and nitrates, performs antimicrobial and anti-inflammatory activities [18], while the peak at 1459.25 cm^{-1} indicates aromatic ring vibrations, suggesting the likely presence of aromatic compounds like flavonoids, that exhibits anti-inflammatory and antioxidant activity [18]. The strong absorption at 1708.98 cm^{-1}, and 1763.32 cm^{-1} corresponds to carbonyl stretching vibrations, indicative of ketones, aldehydes, or esters [15], while the peaks at 2853.28 cm^{-1}, 2922.23 cm^{-1}, and 2953.92 cm^{-1} represent C-H stretching vibrations in aliphatic compounds [19]. The GC-MS result showed that n-hexadecanoic acid (the most abundant compound) exhibits hypocholesterolemic, anti-inflammatory, and antioxidant activity [20]. The 9-Octadecenoic acid (E) has been reported to have anti-inflammatory, anticancer, and hypocholesterolemic benefits as noted by [21]. The 2,4-di-tert-butylphenol exhibits anti-inflammatory, antioxidant, and antibacterial effects [22, 23]. Other studies highlighted the analgesic and anti-inflammatory activity of Tetradecane and Eicosane [24-26]. Similarly, Octacosane possesses anti-inflammatory, antioxidant, and antimicrobial activity, aligning with findings by [27]. The 6, 10, 14-trimethyl-, 2-Pentadecanone, elucidates hypocholesterolemic, anti-inflammatory, and antibacterial effects [28]. According to [29], the Phytol (a diterpene alcohol) has been reported to have antimicrobial, cytotoxic, antioxidant, and anti-inflammatory activities.

Conclusion: Plant species are endowed with several varieties of natural compounds that support their biological activity. The current work aimed to investigate the antioxidant and anti-inflammatory properties and to uncover potential natural compounds in *T. bangwensis*. The present findings suggest that these compounds, 2-Pentadecanone, 6, 10, 14-trimethyl, Octacosane, 2, 4-di-tert-butylphenol, and Phytol, may be responsible for the antioxidant and anti-inflammatory activities. In conclusion, the plant may offer a therapeutic option for treating oxidative and inflammatory diseases.

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