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In vitro Antioxidant Potential of *Hyphaene thebaica* Pulp Bioactive Fraction

Amina Lawal Garba[1,3], Aminu Jibril Sufyan[2], Aisha Abubakar Jinjiri[1], Zainab Bello Musa[1], Nafisa Isiyaka Rabiu[1], Amina Abdulmalik Juda[1], Husna Usman Turadu[1], Fatima AbdulGaffar Nasir[1], Innocent Ojeba Musa[1], Haruna Bala Tsoho[2], Abba Babandi[4] and Hafeez Muhammad Yakasai[1,2]*

[1]Department of Biochemistry, School of Science and Information Technology, Skyline University Nigeria, No. 2 Zaria Road, P.M.B 3076, Kano, Kano State, Nigeria.
[2]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Bayero University Kano, P.M.B 3011, Kano, Kano State, Nigeria.
[3]Integrated Science Department, School of Science Education, Federal College of Education (Technical) Bichi, P.M.B 3473, Bichi, Kano State, Nigeria.
[4]Medical Biochemistry Unit, Faculty of Basic Medical Sciences, Federal University Dutse, P.M.B 7156, Dutse, Jigawa State, Nigeria.

*Corresponding author:
Hafeez Muhammad Yakasai,
Biochemistry Department,
Faculty of Basic Medical Sciences, College of Health Science,
Bayero University Kano,
P.M.B 3011, Kano State,
Nigeria.
Email: hmyakasai.bch@buk.edu.ng

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Abstract

Oxidative stress underlies the development of several chronic disorders, prompting interest in plant-based antioxidants with defined chemical composition and biological efficacy. This study investigated the antioxidant potential of *Hyphaene thebaica* pulp through integrated phytochemical quantification, spectroscopic characterization, chromatographic profiling, and *in vitro* antioxidant assays. The pulp exhibited measurable total phenolic content (19.90 ± 2.20 mg/g) and remarkably high total flavonoid content (615.94 ± 6.36 mg/g), indicating a substantial pool of redox-active metabolites. FTIR analysis of the aqueous fraction revealed characteristic absorption bands corresponding to hydroxyl, carbonyl, aliphatic, aromatic, nitro, and amine functional groups, supporting the presence of phenolics, flavonoids, fatty acids, and related antioxidant-associated constituents. GC–MS profiling identified compounds with reported antioxidant relevance, including oleic acid, tetradecanoic acid, long-chain aliphatic alcohols, and flavone-related structures, suggesting multiple antioxidant mechanisms. Antioxidant assays demonstrated solvent-dependent activity, with ethyl acetate extract showing the strongest DPPH radical scavenging activity (IC_{50} = 4.19 ± 0.92 µg/mL), followed by chloroform and aqueous fractions, while the crude extract exhibited comparatively weaker activity. Total antioxidant capacity further confirmed superior activity of the ethyl acetate fraction (729.16 ± 3.21 µg/mL), indicating enrichment of potent antioxidants in semi-polar solvents. Collectively, the findings suggest that the antioxidant activity of *H. thebaica* pulp arises from the synergistic action of phenolic and flavonoid compounds alongside lipid-derived constituents, providing a mechanistic basis for its potential application as a natural antioxidant source.

INTRODUCTION

Oxidative stress can be considered an imbalances state between the production level of reactive oxygen species (ROS) and the antioxidant defense capacity of an organism, which leads to oxidative damage to lipids, proteins, and DNA [1]. Compounds classified as phenolics, flavonoids, and terpenoids are of great interest in the area of non-enzymatic antioxidants of plant origin because they have multiple mechanisms of action including direct free radical reactions, chelation of transition metal ions, and redox regulation, which are not linked with toxicity observed with synthetic antioxidants [2]. *Hyphaene thebaica* L. Mart (Doum palm) is a common palm prevalent in Africa and the Middle East with a traditional edible and medicinal application. Several solvent extracts of the fruits of *Hyphaene thebaica* have presented potent antioxidant capacities in a number of *in vitro* studies, such as a concentration-dependent increase in radical-scavenging activity in a DPPH radical and ferric reducing

antioxidant power (FRAP) assay, reflecting their content proportions in both extracts [3]. Aqueous and ethanol extracts of the fruits demonstrated a concentration-dependent DPPH radical-scavenging capacity, ensuring the presence of radical-scavenging compounds in the pulp extracts [4]. Phytochemical screenings conducted on the fruit and other plant tissues of *Hyphaene thebaica* have revealed the presence of high levels of polyphenolic compounds, flavonoids, saponins, and other secondary metabolites that have been generally acknowledged to possess antioxidant properties through the scavenging of free radicals, chelation of metal ions, and inhibition of lipid peroxidation reactions in biological systems. Moreover, hot water extracts of *Hyphaene thebaica* fruits presented very strong hydrogen donor capacity, hydroxyl radical-scavenging capacity, and Fe^{2+}-chelating capacity comparable to a common antioxidant standard black tea, despite their lower total content of phenolic compounds, indicating the presence of potent redox-active phytochemicals in the latter [5, 6].

Nevertheless, the nature of the antioxidant properties that solvent-partitioned pulp fractions of *Hyphaene thebaica* possess remains poorly understood in relation to the partitioning of biologically active molecules in fractions of varying polarities. Thus, a broader interpretation that considers the phytochemical composition, functional group chemistry, and chemical profiling of the pulp with respect to antioxidant properties must be carried out.

MATERIALS AND METHODS

Plant sample collection and preparation procedures

A dried ripe *Hyphaene thebaica* fruits were sourced from Kurmi Market, Gwale Local Government, Kano State. It was identified and authenticated at the department of plant Biology, Bayero University, Kano with herbarium number BUKHAN 0380. The fruit was washed thoroughly to remove dirt. The pulp was separated from the seed and pulverized using a mechanical grinder and stored in airtight containers under cool conditions until further use [7].

Extraction of crude extract

Preparation of methanolic extracts described by [8] with slight modification. Exactly, 200 g of the plant powder sample was placed in ultrasonic bath and dissolved with 800 mL of methanol, the extraction was carried out for 40 min at 50 °C, different experimental parameters were designed to optimize the extraction process, such as the liquid–solid ratio, ultrasonic power level (100-200 W), methanol concentration (95%) and extraction time (20-40 min). After extraction, the crude extract was filtered using a filter paper (120 mm, Whatman®, China), and the solvent was evaporated at room temperature. The extracts obtained were kept in a vacuum at optimal temperature until analysis [8].

Solvent-based fractionation using column chromatography

Fractionation of the crude extract was carried out using column chromatography**,** a preparative separation technique based on the differential affinities of compounds for the stationary and mobile phases. The procedure was designed to isolate bioactive fractions of varying polarity by sequential elution with solvents of increasing polarity. A glass chromatography column was packed with silica gel**,** which served as the stationary phase. The column was carefully packed to ensure uniform bed height and avoid air pockets, and pre-conditioned with a non-polar solvent before sample loading.

Approximately 5–10 g of crude plant extract was adsorbed onto a small quantity of silica gel and allowed to dry to form a free-flowing powder. This mixture was gently loaded onto the top of the packed column. The column was then eluted sequentially with solvents of increasing polarity in the following order: n-hexane, a non-polar solvent used to elute lipophilic and hydrocarbon-based compounds (e.g., fats, oils, waxes, terpenes), followed by chloroform, which is moderately polar, ideal for eluting chlorophylls, alkaloids, and other medium-polarity phytochemicals. Ethyl acetate is a polar aprotic solvent that extracts flavonoids, tannins, and phenolic compounds. Similarly, ethanol, a polar aprotic solvent, is effective in eluting glycosides, phenolics, and some sugars. Distilled water was the most polar solvent, used to extract highly polar constituents such as polysaccharides, amino acids, and certain organic acids. Each solvent was applied gradually and in sufficient volume (typically 50–150 mL per stage, depending on column size) to allow thorough elution [9].

Antioxidant assays

Total flavonoids

The total flavonoid content was determined using the method described by [10]. A volume of 0.5 mL of a 2% AlCl solution in ethanol was added to 0.5 mL of the crude extract. After one hour at room temperature, the absorbance was measured at 420 nm using the spectrophotometer. The formation of yellow coloration showed the presence of flavonoids. The samples of the extract were estimated at a final concentration of 1 mg/mL. Using the given equation based on the calibration curve $y = 0.025x, + C$, $R^2 = 0.9812$, where x represented the concentration equivalent of gallic acid (mg/mL).

Total phenolics

The total phenolic content in the extract was determined by the modified Folin-Ciocalteu method [11]. The crude extract (1 mg/mL) was mixed with 5 mL of Folin-Ciocalteu reagent and 4 mL of sodium carbonate. The mixture was vortexed for 15 sec and allowed to stand for 30 min at 40 °C for color development, and absorbance was then measured at 765 nm using a spectrophotometer. Total phenolic content was expressed as mg/mL gallic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, where x represented the concentration equivalent of gallic acid (mg/mL).

DPPH radical scavenging assay

The DPPH antiradical activity of the sample fractions were assayed as reported in literature with slight modifications [12]. Exactly 2 mL of 4 mg/mL sample (crude and the fraction) solution were mixed with 2.0 mL of 0.1 mM freshly prepared DPPH solution (in methanol). After incubation in the dark for 30 min at room temperature, the absorbance of the different sample fractions was measured at 517 nm using ascorbic acid as a positive control.

$$\% \text{ of antioxidant activity} = [(Ac - As) \div Ac] \times 100$$

Where: Ac = Control reaction absorbance; As = Testing specimen absorbance.

Total Antioxidant Capacity (TAC)

TAC was evaluated using the phosphomolybdate method, which was based on the reduction of Mo(VI) to Mo(V) by the antioxidants, forming a green phosphate/Mo(V) complex measurable at 695 nm. In a test tube, 0.3 mL of each fraction (1 mg/mL) was mixed with 3 mL of reagent solution, the reaction mixtures were incubated at 95 °C in a water bath for 90 minutes.

After incubation, the samples were allowed to cool to room temperature. The absorbance of each sample was measured at 695 nm using a spectrophotometer against a blank of reagent solution and solvent. All tests were performed in triplicate, and the results were presented as mean ± standard deviation (SD).

Characterization of *Hyphaene thebaica* bioactive fraction

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR analysis of fractionated extracts of *Hyphaene thebaica* was carried out following the method described by [13] with slight modifications. Approximately 1 mg of each dried extract was finely ground and thoroughly mixed with 100 mg of spectroscopic grade potassium bromide (KBr) in an agate mortar to obtain a homogeneous powder. The mixture was compressed into a translucent disc using a hydraulic press under vacuum. The prepared KBr pellets were scanned in the mid-infrared region (4000–400 cm^{-1}) using an FTIR spectrophotometer (Shimadzu FTIR-8400S or equivalent). The instrument was operated at a resolution of 4 cm^{-1}, and 32 scans were accumulated and averaged to enhance the signal-to-noise ratio. The resulting spectra were analyzed by comparing the absorption bands with standard functional group frequencies to identify the characteristic peaks and corresponding classes of compounds.

Gas chromatography-mass spectrometry (GC-MS) analysis

Separation and identification of compounds were carried out using a Thermo Scientific Trace GC ultra-coupled to an ISQ single quadrupole mass spectrometer. The system was fitted with a TG-5MS fused silica capillary column (30 m × 0.25 mm, 0.1 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. Samples were introduced by injecting 1 µL into the GC injector, with the injector and MS transfer line temperatures maintained at 280 °C. The oven temperature was initially set at 40 °C and held for 3 minutes, then increased at a rate of 5 °C/min to 280 °C, where it was held for a further 5 minutes. The mass spectrometer operated under electron ionization at 70 eV. Chromatograms and spectra were obtained, and quantification of the detected compounds was based on percent relative peak area. Tentative identification of compounds was achieved by comparing their retention times and mass spectra with reference data available in the NIST and Wiley libraries integrated into the GC–MS system [14].

RESULTS

Antioxidant Activity of *Hyphaene thebaica*

Total phenolic and flavonoid contents of *Hyphaene thebaica*

The quantified levels of total phenolic and total flavonoid contents in *Hyphaene thebaica* were detected recorded and expressed as mean values ± standard deviation in (mg/g) of sample *(***Table 1***)*. The total flavonoid content was recorded to be higher (615.94 ± 6.36 mg/g), than flavonoid measured at 19.9 ± 2.2 mg/g**,** reflecting a moderate presence of total phenolics.

Table 1. Total phenolics and flavonoids contents of *Hyphaene thebaica.*

Phytochemicals	Content (mg/g)
Total phenolic	19.90 ± 2.20
Total flavonoid	615.94 ± 6.36

Results are expressed as mean ± S.D, n=3

DPPH (2,2-Diphenyl-1-picrylhydrazy) radical scavenging property of *Hyphaene thebaica* solvent fractions

The antioxidant activity of various *Hyphaene thebaica* (doum palm) fractions was evaluated using IC_{50} values, which represent the concentration required to inhibit 50% of free radical activity (**Fig. 1**). Among the tested fractions, the ethyl acetate fraction exhibited the strongest antioxidant activity with an IC_{50} of 4.19 ± 0.92 µg/mL. This was followed by the chloroform (5.22 ± 0.13 µg/mL) and aqueous (5.57 ± 0.12 µg/mL) fractions, both of which demonstrated notable radical-scavenging capacities. The ethanol extract showed moderate activity with an IC_{50} of 7.60 ± 0.53 µg/mL, whereas, the n-hexane fraction had the highest IC_{50} value among the solvent fractions (9.24 ± 0.10 µg/mL). The crude extract, with an IC_{50} of 27.66 ± 0.35 µg/mL, showed the least DPPH radical scavenging activity. As expected, the gallic acid standard showed the most potent activity with an IC_{50} of 0.0001 ± 0 µg/mL, serving as a benchmark for comparison.

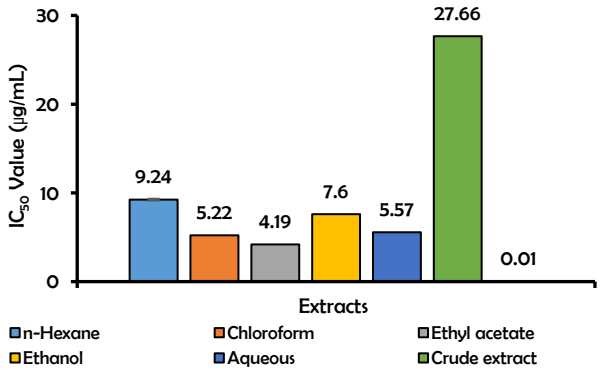

Fig 1. Scavenging activity of *Hyphaene thebaica* against DPPH radical. Results are expressed as mean ± S.D.

Total antioxidant activity (TAC) of *Hyphaene thebaica*

The total antioxidant capacity (TAC) of *Hyphaene thebaica* fractions obtained using different solvents expressed in µg/mL was presented in **Fig. 2**. The result shows that ethyl acetate fraction revealed the highest TAC of 729.16 ± 3.21 µg/mL, followed by n-hexane, chloroform, ethanol and water, respectively. The crude extract exhibited the lowest antioxidant capacity, with a significantly lower ($p<0.05$) value of 42.60±8.37 µg/mL compared to all other fractions.

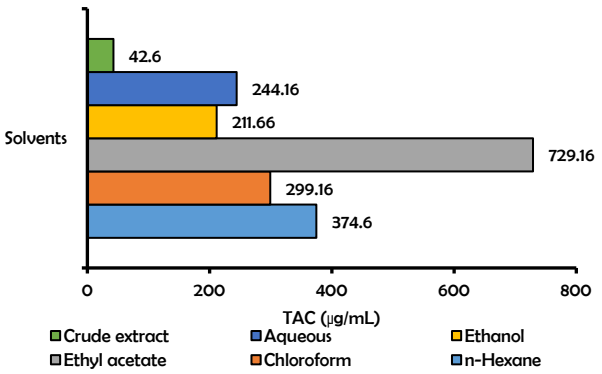

Fig. 2. Total Antioxidant Capacity (TAC) of *Hyphaene thebaica*. Results are expressed as mean ± S.D.

Fourier Transform Infrared (FTIR) of Aqueous Fraction of *Hyphaene thebaica*

Table 2 presented the FTIR absorbance data. A strong absorbance at 3268 cm^{-1} was attributed to the stretching vibration of the –C≡CH group, indicating the presence of alkynes. At 2933 cm^{-1}, a medium intensity C–H stretch is observed, which is characteristic of alkanes, signifying saturated hydrocarbons. A

strong absorption at 1722 cm^{-1}corresponds to the C=O stretching of esters, suggesting the presence of carbonyl-containing ester groups. Two strong peaks at 1525 cm^{-1} and 1346 cm^{-1} are due to N–O stretching vibrations, which confirm the presence of nitro compounds. The peak at 1253 cm^{-1} shows a medium intensity C–H wag typically found in alkyl halides, especially where halogens are bonded to methylene groups (–CH_2X). At 1030 cm^{-1}, a medium C–N stretch suggests the presence of aliphatic amines, indicating nitrogen-containing functional groups. A medium absorption at 922 cm^{-1} corresponds to O–H bending, typically found in carboxylic acids, showing the presence of acidic groups. A strong peak at 899 cm^{-1} is attributed to C–H vibrations in aromatic alkanes, pointing to the substitution on an aromatic ring (**Fig. 3)**. Lastly, a medium absorption at 821 cm^{-1} represents the C–Cl stretch, confirming the presence of alkyl halides (specifically chlorinated compounds).

Table 2. FTIR identified functional groups in ethyl acetate fraction of *Hyphaene thebaica.*

Absorbance (cm^{-1})	Class of compound	Functional group	Intensity
3268	-C≡CH-H:CH Stretch	Alkynes	Strong
2933	C-H Stretch	Alkanes	Medium
1722	C≡O Stretch	Esters	Strong
1525	N-O Stretch	Nitro compounds	Strong
1346	N-O Stretch	Nitro compounds	Strong
1253	C-H wag (-CH_2X)	Alkyl halides	Medium
1030	C-N Stretch	Aliphatic Amines	Medium
922	O-H bend	Carboxylic acids	Medium
899	C-H	Aromatic Alkanes	Strong
821	C-Cl Stretch	Alkyl halides	Medium

Results are expressed as mean ± S.D, n= 3

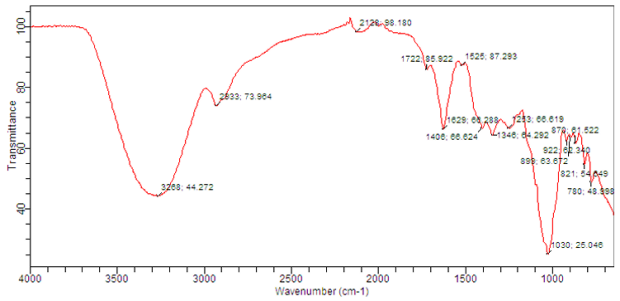

Fig. 3. The Fourier Transform Infrared of ethyl acetate fraction.

GC-MS profiling of ethyl acetate fraction of *Hyphaene thebaica*

GC–MS profiling of the ethyl acetate fraction of *Hyphaene thebaica* pulp revealed multiple phytoconstituents measured by retention time and peak area (**Table 3)**. Among these, compounds with previously reported or plausible antioxidant relevance were identified. The major fatty acid component oleic acid was detected at RT 15.8 min with high relative abundance, indicating a significant presence in the ethyl acetate fraction. Saturated fatty acid tetradecanoic acid (myristic acid) was observed at RT 13.9 min. Additionally, 15-hydroxypentadecanoic acid was present at RT 16.3 min. Aromatic derivatives including 1,3-benzenedicarboxylic acid, 5-(1,1-dimethylethyl) (RT 23.7 min) and a benzopyranone derivative 4H-1-benzopyran-4-one, 5,7-dimethoxy-2-phenyl (RT 24.7 min) were identified (**Figs. 4** to **8**). These constituents represent chemical classes commonly observed in plant extracts and may contribute to the bioactivity of the fraction, particularly its antioxidant potential.

Table 3. GC-MS profile of ethyl acetate fraction of *Hyphaene thebaica.*

Peak	Retention Time (RT)	Area (%)	Compounds	Molecular Weight (g/mol)
1	13.9	6082155.21	Tetradecanoic acid (Myristic acid)	228.37
2	15.8	4682958.68	Oleic acid	282.46
3	16.3	483081.1	15-Hydroxypentadecanoic acid	258.40
4	23.7	530512.71	1,3-Benzenedicarboxylic acid, 5-(1,1-dimethylethyl)	222.24
5	24.7	1886076.63	4H-1-Benzopyran-4-one, 5,7-dimethoxy-2-phenyl	282.29

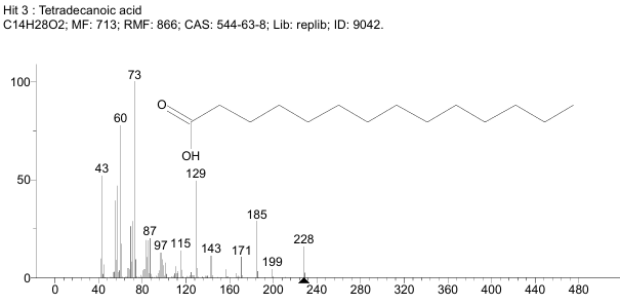

Fig. 4. Tetradecanoic acid, retention time of 13.9 minute.

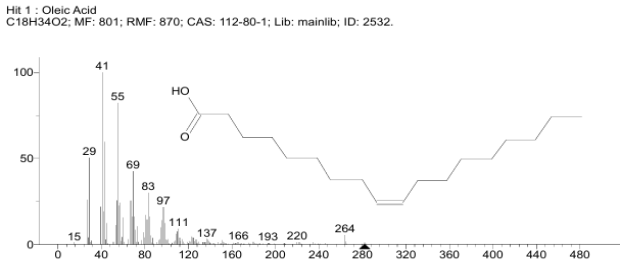

Fig. 5. Oleic acid, retention time of 15.8 minute.

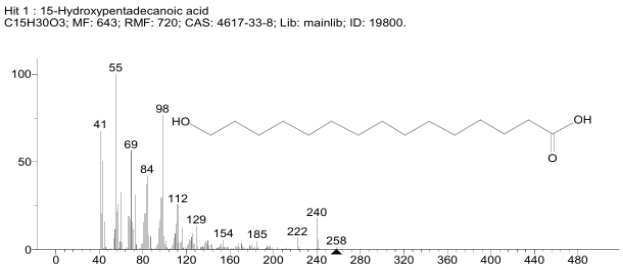

Fig. 6. 15-Hydroxypentadecanoic acid, retention time of 16.3 minute.

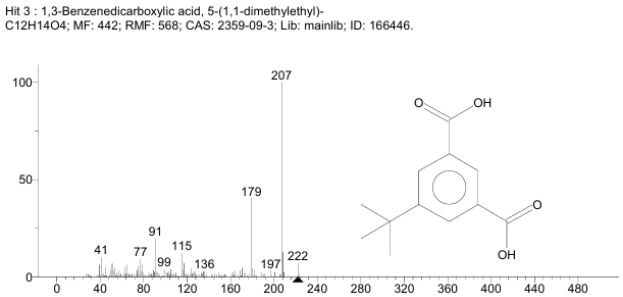

Fig. 7. 1,3-Benzenedicarboxylic acid, 5-(1,1-dimethylethyl) retention time of 23.7 minute.

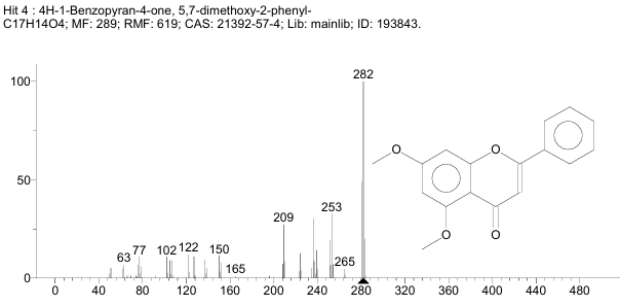

Fig. 8. 4H-1-Benzopyran-4-one, 5,7-dimethoxy-2-phenyl, retention time of 24.7 minute.

DISCUSSION

The antioxidant effects observed in this study can be attributed to the phytochemical composition of *Hyphaene thebaica* pulp and the known redox behavior of plant-derived bioactive. The total phenolic content measured in *Hyphaene thebaica* pulp (19.90 ± 2.20 mg/g) and total flavonoid content (615.94 ± 6.36 mg/g) suggest a phytochemical fingerprint strongly linked with antioxidant properties. Amongst antioxidants in plants, polyphenols have emerged as most potent because they can readily donate hydrogen or electron equivalents, form complexes with redox-active metal ions, and delocalize excess charge in free radicals via resonance phenomena [15]. A considerable amount of literature confirms that plant extracts with higher concentrations of polyphenols have better radical scavenging properties and reducing capacity, thus, establishing the importance of total phenolic content measured in this study [16,17].

The significantly high flavonoids content in the pulp of *Hyphaene thebaica*, strongly suggest that flavonoids make up a major fraction of its total phenolic constituents. Flavonoids have been widely recognized for their excellent antioxidant activity resulting from their specific molecular structures including hydroxyl substitution on the B-ring and A-C rings combination [18]. Higher levels of flavonoids have been observed to have a direct positive correlation with antioxidant activity in DPPH, ABTS, and FRAP assays in a wide variety of plant samples, thus making it a major contributor towards antioxidant capacity in most samples [19,20]. Similar observations have previously been reported in the fruit extracts of *Hyphaene thebaica*, where high levels of phenolic and flavonoid compounds with good free radical-scavenging abilities and metal ions chelating activity [21]. The antioxidant activity in the pulp of *Hyphaene thebaica* can thus primarily be attributed to the presence of these phenolic compounds. Additionally, the high level of flavonoids can also improve antioxidant activity. The antioxidant activity can be attributed to synergistic effects among different phytochemical components, including fatty acids and aromatic compounds, which have already been detected in such plant extracts [22].

Furthermore, the antioxidant potential of *Hyphaene thebaica* extracts was assessed using IC_{50} values, where lower values indicate stronger free radical scavenging activity. Among all the tested fractions, ethyl acetate fraction showed the most potent antioxidant activity with an IC_{50} of 4.19 ± 0.92 µg/mL**,** suggesting it contains highly active phenolic or flavonoid compounds. This was followed by the chloroform (5.22 ± 0.13 µg/mL) and aqueous fraction (5.57 ± 0.12 µg/mL)**,** both of which also demonstrated significant antioxidant capacities, possibly due to the presence of moderately polar phytochemicals. The ethanol fraction exhibited moderate activity **(**7.60 ± 0.53 µg/mL**),** reflecting the fraction ability to solubilize a broader range of both polar and non-polar constituents, which may dilute the concentration of potent antioxidants.

The n-hexane fraction**,** being non-polar, showed the least antioxidant activity among the fractions **(**9.24 ± 0.10 µg/mL**),** likely due to its limited ability to extract polar antioxidant compounds such as phenolics. The crude extract displayed the highest IC_{50} value (27.66 ± 0.35 µg/mL)**,** indicating relatively weak antioxidant activity, possibly because of the presence of both active and inactive components, which may reduce the overall efficacy. The standard compound, gallic acid, had an IC_{50} of 0.001 ± 0 µg/mL, highlighting its exceptional antioxidant potency. Further studies using different solvents and plant parts also support these findings. For example, ethyl acetate fruit extract showed an IC_{50} of 52.21 µg/mL [23]**,** while distilled water extracts of the fruit and seed had IC_{50} values of 31.82 ± 0.05 µg/mL and 26.07 ± 0.03 µg/mL [24]**,** respectively. Notably, the ethanol extract of the fruit mesocarp and epicarp demonstrated exceptional antioxidant activity with IC_{50} values of 0.078 ± 0.02710 µg/mL and 0.005 ± 0.003 µg/mL [25]**,** respectively surpassing even standard antioxidants in potency. Additionally, FRAP (Ferric Reducing Antioxidant Power) assays revealed strong reducing capacities in ethanol and hexane fruit extracts, with values of 13.571 µmol and 7.658 µmol [26]**,** respectively. DPPH results for the hexane fruit extract showed a moderate activity of 42.762 µmol [26]**.**

The Fourier transform infrared spectroscopy (FTIR) spectrum of the ethyl acetate fraction of *Hyphaene thebaica* pulp shows several absorption peaks attributable to different functional groups common in phytoconstituents known to possess biological activity. The broad absorption peak at 3268 cm^{-1} corresponds to O-H bonds, which are attributed to hydroxyl moieties in phenolic compounds and alcohols. The presence of hydroxyl moieties in these compounds is attributed to their antioxidant capacity based on hydrogen donation and radical reactions [17,19]. The absorption band at 2933 cm^{-1}, which corresponds to the stretching of aliphatic C-H bonds, is an indication of alkanes, which can be seen in fatty acids. Even though aliphatic C-H bonds do not have a direct role in radical scavenging reactions, their presence with other polar functions in a molecule can sometimes show intricate molecular structures in plant extracts that influence oxidative stability and lipid peroxidation behavior [22,27].

The strong absorption peak observed at 1722 cm^{-1} corresponds to C=O bonds in esters and compounds with carboxyl groups, indicating the presence of fatty acids, esters, and other phytochemicals with carbonyl functions. Carbonyls are usually found in organic acids and lipids with potential indirect roles in antioxidant defense mechanisms in relation to modulation of lipid peroxide production and reactions with reactive oxygen species [22]. The appearance of distinct bands at 1525 cm^{-1} and 1346 cm^{-1}, which correspond to the stretching vibrations of nitrogen–oxygen bonds, points to the presence of nitro group-containing or nitrogen-associated compounds. Although nitro groups are not considered characteristic antioxidants in a classical sense, nitrogen-containing compounds in plant biomass can be part of a heterocycle or a conjugate with redox properties, especially when co-occurring with phenolic compounds [15].

The absorptions observed at 1253 cm^{-1} and 821 cm^{-1}, which can be attributed to C–H wagging and C–Cl stretching in alkyl halides, represent a sign of aliphatic or aromatic substitution. Such moieties are frequently found in FTIR analysis of intricate

plant extracts and can represent a naturally occurring halogenated metabolite, which can arise because of environmental exposure but are not generally considered key contributors in terms of antioxidant capacity but rather a structurally supportive function [28].

Moreover, the presence of a band at 1030 cm^{-1}, attributed to C-N stretching in aliphatic amines, indicates the presence of nitrogenous phytochemicals with potential ancillary roles in antioxidant defences, such as electron donation and reactions with reactive intermediates [29]. Additionally, the presence of a medium-intensity absorption peak at 922 cm^{-1}, attributed to O-H bending in carboxylic acids, reinforces this observation, thus indicating the presence of carboxylic acids and phenolic compounds in the ethyl acetate fraction. The presence of a strong absorption peak at 899 cm^{-1}, which corresponds to the C-H bonds of aromatic rings, further supports the presence of an aromatic compound, which is important in the antioxidant mechanism because of its delocalized π electrons [19].

The ethyl acetate fraction of *Hyphaene thebaica* pulp contained several compounds with potential antioxidant activity. Oleic acid, a monounsaturated fatty acid, has been shown in both *in vitro* and *in vivo* models to mitigate oxidative stress. It can reduce lipid peroxidation and modulate endogenous antioxidant defences under oxidative conditions, suggesting that fatty acids like oleic acid may play supportive roles in the overall antioxidant capacity of plant extracts [30]. Evidence from seed oils rich in oleic acid also points to radical-modulation and lipid protection effects, consistent with its presence in antioxidant-active fractions of plant extracts [31].

Saturated fatty acids such as tetradecanoic acid (myristic acid) were also detected. Although saturated fatty acids are not classic radical scavengers, their presence in antioxidant evaluations of plant oils and extracts has been associated with overall oxidative stability and nutraceutical properties in some studies. Oils with balanced fatty acid profiles, including both saturated and unsaturated lipids, often exhibit measurable antioxidant activity in chemical assays, attributed to complex interactions among constituents [32].

The identification of substituted aromatic carboxylic acid derivatives such as 1,3-benzenedicarboxylic acid and benzopyranone scaffolds like 4H-1-benzopyran-4-one, 5,7-dimethoxy-2-phenyl is noteworthy. Aromatic carboxylic acids and benzopyranone cores resemble structural motifs found in known phenolic antioxidants, where conjugated systems and functional substituents enhance radical stabilization and electron donation. Compounds with benzopyranone or flavone-like structures frequently show significant free radical scavenging in DPPH, ABTS, and related assays, though specific activity depends on substitution patterns. This structural relevance aligns with observations in other plant extracts where related scaffolds contribute to antioxidant profiles [33]. Hydroxy fatty acids like 15-hydroxypentadecanoic acid may influence oxidation processes in lipid systems. Hydroxyl functional groups can interact with radical species and metal ions, potentially affecting lipid peroxidation pathways, although direct antioxidant assays for this compound are limited [34]. The presence of such molecules in combination with more studied antioxidants could contribute to synergistic effects in complex mixtures.

The ethyl acetate fraction of *Hyphaene thebaica* pulp, thus exhibits a blend of fatty acids and aromatic compounds with plausible antioxidant relevance. These findings complement previous reports that *Hyphaene thebaica* extracts show significant antioxidant activity *in vitro*, correlating with the presence of phytochemicals including fatty acids, phenolic compounds, and other redox-active constituents [23].

CONCLUSION

The findings of this study demonstrate that *Hyphaene thebaica* pulp possesses notable antioxidant potential, supported by its appreciable phenolic content, exceptionally high flavonoid concentration, and diverse antioxidant-associated functional groups and metabolites. FTIR and GC–MS analyses revealed the presence of phenolics, flavonoids, fatty acids, and related compounds that contribute to redox activity through free radical scavenging, metal chelation, and stabilization of lipid systems. The solvent-dependent antioxidant responses, particularly the strong DPPH scavenging and total antioxidant capacity observed in the ethyl acetate fraction, highlight the selective enrichment of bioactive antioxidants in semi-polar extracts. The bioactivity evidence provides a mechanistic basis for the antioxidant properties of *Hyphaene thebaica* pulp and supports its potential use as a natural antioxidant source in functional food and nutraceutical applications.

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