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PHYTOCHEMICAL PROFILING, TOTAL PHENOLIC AND FLAVONOID CONTENTS, AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS FROM FIVE SUDANESE MEDICINAL FRUITS

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ABSTRACT

Background: In the Republic of Sudan, approximately 90% of the populace relies upon indigenous medicinal flora as their principal source of primary health care. Nevertheless, comprehensive scientific documentation regarding the chemical composition and pharmacological attributes of numerous locally consumed fruit species remains conspicuously deficient. **Objective:** The present investigation was undertaken to systematically evaluate the phytochemical constitution, quantify total phenolic and flavonoid pools, and assess the free radical-neutralizing capacity of ethanolic extracts derived from five traditionally utilized Sudanese fruits: Ceratonia siliqua (carob), Grewia tenax (guddaim), Adansonia digitata (baobab), Hibiscus sabdariffa (roselle), and Tamarindus indica (tamarind). **Methods:** Desiccated plant materials underwent exhaustive maceration with 80% ethanol. Preliminary phytochemical reconnaissance was executed employing established qualitative assay protocols. Total phenolic content (TPC) and total flavonoid content (TFC) were spectrophotometrically quantified. Antioxidant efficacy was appraised via the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, while bioactive constituent profiling was accomplished through Gas Chromatography-Mass Spectrometry (GC-MS). **Results:** T. indica yielded the highest extraction recovery (71.0%), followed by H. sabdariffa (47.28%). Phytochemical screening confirmed the ubiquitous presence of flavonoids, tannins, terpenoids, coumarins, and saponins across most specimens. C. siliqua demonstrated the most elevated TPC (1149.37 µg GAE/g dry extract) and TFC (561.36 µg CAE/g dry extract). DPPH radical scavenging activity was maximal for C. siliqua (80.0% RSA), closely trailed by H. sabdariffa (78.0% RSA) and A. digitata (76.0% RSA). GC-MS chromatographic analysis unveiled signature bioactive markers, prominently alpha-methyl mannofuranoside (51.26%) in C. siliqua, 5-hydroxymethylfurfural (30.39%) in T. indica, and sucrose (19.15%) in G. tenax. **Conclusion:** These empirical findings substantiate that the examined Sudanese fruits constitute substantial repositories of phenolic bioactives and manifest considerable antioxidant prowess, thereby providing scientific corroboration for their ethnomedicinal utilization as functional food matrices and nutraceutical formulations.

KEYWORDS: Sudanese medicinal flora, secondary metabolites, total phenolics, DPPH assay, GC-MS metabolomics.

INTRODUCTION

The therapeutic deployment of botanical agents represents a cornerstone of healthcare delivery systems throughout the African continent, with epidemiological evidence indicating that approximately 80% of the regional demographic actively incorporates plant-derived remedies into their disease prevention and management strategies.[1] This profound reliance upon phytotherapy is particularly conspicuous in Sudan, a nation distinguished by remarkable ecogeographic diversity—traversing hyperarid Saharan zones in the northern latitudes to seasonally inundated tropical rainforest ecosystems in the southern territories—which collectively harbor an estimated 3,156 indigenous vascular plant species, of which approximately 15% are endemic to the region.[2] The sociocultural fabric of Sudanese society remains intimately interwoven with traditional medicinal practices; indeed, over 90% of the citizenry—especially inhabitants of rural and peri-urban communities—continue to depend upon indigenous plant-based therapeutics as their primary recourse for addressing a broad spectrum of health afflictions.[3]

Among the pantheon of ethnobotanically significant Sudanese flora, five fruit-bearing species have achieved exceptional prominence: Ceratonia siliqua L. (carob), Grewia tenax (Forssk.) Fiori (guddaim), Adansonia digitata L. (baobab), Hibiscus sabdariffa L. (roselle), and Tamarindus indica L. (tamarind). These botanical commodities are conventionally ingested either in their freshly harvested state, following desiccation and dehydration, or subsequent to culinary processing into traditional beverages, fermented concoctions, and nutrient-dense porridges. The enduring popularity of these fruits is attributable to their dual functionality as both nutritional supplements and medicinal adjuncts, with documented applications in the management of gastrointestinal disturbances, febrile conditions, cardiovascular pathologies, and metabolic dysfunctions.[4,5]

The pharmacotherapeutic efficacy of these plant matrices is largely attributable to their sophisticated secondary metabolic apparatus, which engenders an extensive repertoire of bioactive phytoconstituents—preeminently phenolic acids, flavonoid glycosides, and condensed tannins. These compounds are widely recognized for their multifaceted biological activities, encompassing potent free radical scavenging, broad-spectrum antimicrobial efficacy, and significant anti-inflammatory modulation.[6,7] Phenolic compounds, in particular, exert their antioxidant effects through multiple mechanistic pathways, including direct radical neutralization, transition metal chelation, and enzymatic modulation of oxidative stress-responsive signaling cascades.[8]

Notwithstanding their pervasive traditional utilization, there persists a conspicuous lacuna in the systematic phytochemical characterization of Sudanese specimens of these species. Specifically, comprehensive investigations that concurrently quantify major phenolic markers, evaluate in vitro antioxidant capacity through validated colorimetric assays, and corroborate findings with confirmatory advanced analytical techniques—such as Gas Chromatography-Mass Spectrometry (GC-MS)—remain critically underrepresented in the contemporary scientific corpus. Such integrated metabolomic profiling is essential not only for validating ethnopharmacological claims but also for establishing quality control parameters essential for the standardization of botanical products intended for nutraceutical applications.

Accordingly, the present investigation was designed with the following specific objectives:

1-To determine the gravimetric extraction yield of ethanolic macerates prepared from five indigenous Sudanese fruits.
2-To conduct preliminary qualitative screening for major phytochemical classes.
3-To spectrophotometrically quantify total phenolic and total flavonoid content.
4-To evaluate the in vitro free radical scavenging capacity employing the DPPH assay.
5-To identify volatile and semi-volatile bioactive constituents through GC-MS chromatographic analysis.

MATERIALS AND METHODS

Sample Collection and Preparation

Mature, disease-free fruits of C. siliqua, G. tenax, A. digitata, H. sabdariffa, and T. indica were systematically collected from geographically distinct regions across Sudan to ensure representativeness of intraspecific phytochemical variability. Taxonomic authentication was performed by expert botanists at the Department of Botany, University of Khartoum, where voucher specimens were deposited for reference. Collected plant materials were subjected to ambient air-drying under controlled conditions (25 ± 2°C, relative humidity 45–50%) until constant weight was achieved. Dried specimens were subsequently pulverized to a fine homogenous powder utilizing a mechanical grinder, sieved through a 40-mesh screen, and stored in hermetically sealed amber glass containers at ambient temperature, protected from light and moisture pending extraction.

Chemicals and Reagents

All reagents and chemicals utilized in this investigation were of analytical reagent grade. Folin-Ciocalteu phenol reagent, gallic acid, sodium carbonate (anhydrous), aluminum chloride hexahydrate, sodium nitrite, sodium hydroxide, and DPPH (2,2-diphenyl-1-picrylhydrazyl) were sourced from Sigma-Aldrich (St. Louis, MO, USA). Methanol and absolute ethanol were procured from Merck (Darmstadt, Germany). Deionized water (resistivity ≥18.2 MΩ·cm) was generated using a Milli-Q Advantage A10 water purification system (Millipore, Billerica, MA, USA).

Preparation of Extracts

The extraction protocol was performed according to the methodology described by Sukhdev et al.[9], with minor modifications. In brief, 100 g of powdered plant material from each species was separately subjected to exhaustive maceration in 1 L of 80% aqueous ethanol at ambient temperature for 7 days, with intermittent manual agitation (three times daily). The solvent was replenished daily with fresh 80% ethanol (500 mL) following vacuum filtration through Whatman No. 1 filter paper to ensure complete extraction. The combined filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator (Büchi Rotavapor R-200) until complete solvent removal was achieved. The resultant dried extracts were weighed gravimetrically, transferred to pre-weighed vials, and stored at 4°C in a desiccator pending subsequent analyses. The extraction yield (w/w) was calculated using the following formula:

Yield\ (\%) = \frac{Weight\ of\ dried\ extract}{Weight\ of\ initial\ powdered\ sample} \times 100

Phytochemical Screening

Qualitative phytochemical screening was conducted employing standard protocols as described by Sofowora[10] and Harborne.[11] The crude ethanolic extracts were systematically evaluated for the presence of the following secondary metabolite classes: alkaloids (Dragendorff's and Mayer's test), flavonoids (Shinoda test with magnesium and hydrochloric acid), tannins (ferric chloride test and gelatin precipitation), saponins (foam formation and hemolytic activity), steroids (Salkowski reaction with concentrated sulfuric acid), triterpenoids (Liebermann-Burchard test), coumarins (fluorescence under UV light following ammonia vapor exposure), and anthraquinones (Bornträger's test). Results were scored semi-quantitatively as either absent (−), present in trace amounts (+), moderate abundance (++), or high abundance.(+++)

Total Phenolic Content (TPC)

Total phenolic content was spectrophotometrically quantified utilizing the Folin-Ciocalteu colorimetric method, as adapted from Ainsworth and Gillespie.[12] A calibration curve was constructed using serial dilutions of gallic acid (0–100 mg/L). Briefly, 25 µL of extract solution (1 mg/mL in methanol) or gallic acid standard was dispensed into a 96-well microplate, followed by the addition of 50 µL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water). After thorough mixing for 20 seconds, 200 µL of sodium carbonate solution (700 mM) was added, and the reaction mixture was incubated in the dark at ambient temperature for 2 hours to allow for complete color development. Absorbance was recorded at 765 nm using an Epoch 2 microplate reader (BioTek Instruments, Winooski, VT, USA). TPC values were expressed as micrograms of gallic acid equivalents per gram of dry extract (µg GAE/g dry extract), calculated from the regression equation of the standard curve.

Total Flavonoid Content (TFC)

Total flavonoid content was determined employing the aluminum chloride colorimetric assay according to Singh et al.[13], using catechin as the reference standard. For each sample, 0.25 mL of extract (1 mg/mL in methanol) was combined with 0.75 mL of methanol in a test tube. Subsequently, 75 µL of 5% sodium nitrite solution was added, and the mixture was allowed to react for 6 minutes. Following this, 150 µL of 10% aluminum chloride hexahydrate solution was introduced, and the reaction was allowed to proceed for an additional 5 minutes. Finally, 0.5 mL of 1.0 M sodium hydroxide solution was added, and the total volume was adjusted to 2.5 mL with deionized water. Absorbance was measured spectrophotometrically at 510 nm against a reagent blank. Results were expressed as micrograms of catechin equivalents per gram of dry extract (µg CAE/g dry extract) based on a catechin calibration curve (0–200 mg/L).

DPPH Radical Scavenging Activity

The free radical scavenging capacity was evaluated using the DPPH assay, as described by Shimada et al.[14] with appropriate modifications. DPPH radical solution (300 µM in ethanol) was freshly prepared and stored in the dark at 4°C until use. In a 96-well microplate, 100 µL of varying concentrations of extract solution (500 µg/mL final concentration) were mixed with 100 µL of DPPH solution, yielding a final reaction volume of 200 µL. The plate was incubated at 37°C for 30 minutes in the dark to allow for complete reaction. Absorbance was measured at 517 nm using a microplate reader. Propyl gallate (500 µg/mL) was employed as a positive reference standard. A control sample (100 µL of solvent instead of extract) was processed identically. The radical scavenging activity (RSA) was calculated using the following formula:

RSA (%) = [(A_control − A_sample) / A_control] × 100

GC-MS Analysis

The identification of volatile and semi-volatile bioactive constituents was accomplished using a Shimadzu GC/MS-QP2010 Ultra system (Shimadzu Corporation, Kyoto, Japan) equipped with an Rtx-5ms fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Restek Corporation, Bellefonte, PA, USA). Helium (purity ≥99.999%) was employed as the carrier gas at a constant flow rate of 1.61 mL/min. The temperature program was as follows: initial column temperature of 60°C, held for 2 minutes, then ramped at 10°C/min to 300°C, with a final isothermal hold of 5 minutes. The injection port temperature was maintained at 250°C, and the injection volume was 1 µL with a split ratio of 10:1. The mass spectrometer was operated in electron impact ionization mode (70 eV) with scan acquisition over the mass range m/z 40–250. Compound identification was achieved by comparing acquired mass spectra with reference spectra from the National Institute of Standards and Technology (NIST) Mass Spectral Library (Version 2017). Relative percentage abundance

of each identified component was calculated by integrating the peak areas in the total ion chromatogram.

Statistical Analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard deviation (SD). Data processing and statistical computations were performed using Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA).

RESULTS

Extraction Yield

The gravimetric extraction yields demonstrated substantial interspecific variability, with percentages ranging from 29.0% to 71.0% (Table 1). T. indica exhibited the most substantial recovery (71.0%), followed by H. sabdariffa (47.28%) and A. digitata (31.0%). The most modest yields were recorded for C. siliqua (29.0%) and G. tenax (29.56%).

Table 1: Percentage yield of ethanolic extracts from five Sudanese fruits.

Sample	Weight of sample	Weight of extract	Yield %
Grewia tenax	100	26.67	29.56
Ceratonia siliqua L.	100	29	29
Tamarindus indica linn	100	71	71
Adansonia digitata L.	100	31	31
Hibiscus Sabdariffa L.	100	43.48	47.28

Phytochemical Screening

Qualitative phytochemical analysis revealed a diverse array of secondary metabolites across the five extracts (Table 2). Flavonoids, coumarins, triterpenoids, and anthraquinones were ubiquitously distributed. Saponins were detectable in G. tenax, T. indica, and A. digitata, yet conspicuously absent from C. siliqua and H. sabdariffa. Tannins were present in all extracts with the sole exception of T. indica. Notably, alkaloids and steroids were uniformly undetectable across all investigated samples.

Table 2: Phytochemical screening of ethanolic extracts.

Sample	Saponin	Cumarin	Alkaloids	Flavonoids	Tannins	Steroids	Triterpens	Anthraquinone
Grewia tenax	+	++	_	+	+	_	++	_
Ceratonia siliqua L.	_	+	_	+	+	_	+	+++
Tamarindus indica linn	+	+	_	+	_	_	+	_
Adansonia digitata L.	++	+	_	+	+	_	++	_
Hibiscus Sabdariffa L.	_	+	_	+	+	_	++	+

Key: + = Trace, ++ = Moderate, +++ = High abundance, − = Not detected

Total Phenolic and Flavonoid Contents

The quantitative determination of phenolic and flavonoid pools revealed significant species-dependent variation (Table 3). C. siliqua exhibited the most elevated TPC (1149.37 µg GAE/g dry extract), closely followed by A. digitata (1096.26 µg GAE/g dry extract) and H. sabdariffa (1042.48 µg GAE/g dry extract). In contrast, T. indica displayed the lowest TPC (382.26 µg GAE/g dry extract). A parallel trend was observed for TFC, with C. siliqua registering the highest concentration (561.36 µg CAE/g dry extract) and T. indica the lowest (87.75 µg CAE/g dry extract). Notably, H. sabdariffa exhibited extraordinarily elevated total tannin content (113,542.16 µg CAE/g dry extract).

Table 3: Total phenolic, flavonoid, and tannin contents of ethanolic extracts.

Sample	Total phenolic contents (µg of CAE/g dry extract)	Total Flavonoid contents (µg of CAE/gdry extract)	Total Tannin contents (µg of CAE/g dry extract)
Grewia tenax	759.15±0.07	88.58±0.00	15317.16±0.00
Ceratonia siliqua L.	1149.37±0.2	561.36±0.04	11997.16±0.33
Tamarindus indica linn	382.26±0.04	87.75±0.00	14952.16±0.04
Adansonia digitata L.	1096.26±0.05	290.52±0.00	11873.83±0.02
Hibiscus Sabdariffa L.	1042.48±0.6	396.08±0.00	113542.16±0.07

Values expressed as mean ± SD (n = 3)

Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging activity, evaluated at the maximal concentration of 500 µg/mL, is presented in Table 4. C. siliqua demonstrated the most potent antioxidant efficacy (80.0% RSA), which was comparable to that of H. sabdariffa (78.0%) and A.

digitata (76.0%). T. indica (59.0%) and G. tenax (57.0%) exhibited comparatively attenuated radical neutralizing capacity. The reference antioxidant, propyl gallate, displayed 89.0% RSA under identical assay conditions.

Table 4: DPPH radical scavenging activity of ethanolic extracts.

Sample	% RSA± SD (DPPH)
Grewia tenax	57±0.004
Ceratonia siliqua L.	80±0.002
Tamarindus indica linn	59±0.02
Adansonia digitata L.	76±0.002
Hibiscus Sabdariffa L.	78±0.001
Standard (Propyl gallate)	89± 0.01

Values expressed as mean ± SD (n = 3)

GC-MS Analysis of Bioactive Compounds

Comprehensive GC-MS metabolomic profiling revealed distinctive phytochemical signatures for each species, with major identified constituents summarized in the following tables.

Table 5: Phytocomponents identified in Ceratonia siliqua ethanolic extract.

Peak#	Name	R.Time	Area%
1	Glyceraldehyde	4.479	1.09
2	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	4.669	0.41
3	5-Hydroxymethylfurfural	5.459	4.38
4	1H-Azonine,octahydro-1-nitroso-	5.676	1.08
5	2- Pentene,2,3,4-trimethyl	6.738	1.45
6	2- Furanmethanol, tetrahydro-	6.920	1.08
7	Sucrose	7.469	9.02
8	Alpha,-Methyl mannofuranoside	9.664	51.26
9	2H-Indol-2-one,1-(2,6-dichlorophenyl)-1,3-di	14.663	28.02
10	9-Octadecenamide,(z)-	16.177	2.21
	Total		100

Table 6: Phytocomponents identified in Grewia tenax ethanolic extract.

Peak#	Name	R.Time	Area%
1	Glycerin	2.862	11.87
2	Glyceraldehyde	4.492	10.90
3	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	4.674	2.92
4	5-Hydroxymethylfurfural	5.467	1.63
5	1,2,3-Propanetriol,1-acetate	5.595	4.93
6	2-Propanol,1-propoxy-	5.842	4.04
7	Heptanoic acid,6-oxo-	5.916	3.57
8	Butanoic acid,3-hydroxy-3methyl-	6.527	1.47
9	Sucrose	7.470	19.15
10	1-Dodecanol	7.781	1.00
11	Cycloheptasiloxanose, tetradecmethyl-	7.914	0.72
12	D-erthro-Pentose,2-deoxy-	8.787	4.76
13	beta-D-Glucopyranose,-O-beta-D-galactor	9.050	11.00
14	d-Mannitol,1,4-anthydro-	9.313	2.19
15	Hexasiloxane,tetradecamethyl-	9.358	0.94
16	Ethanol ,2-(dodecyloxy)-	9.933	1.91
17	Heptasiloxane tetradecamethyl-	10.896	0.86
18	Heptasiloxane tetradecamethyl-	12.425	0.99
19	Hexasiloxane,tetradecamethyl-	13.887	1.39
20	Heptasiloxane tetradecamethyl-	15.235	1.75
21	9-Octadecenamide,(z)-	16.175	2.26
22	Heptasiloxane tetradecamethyl-	16.508	2.64
23	Heptasiloxane tetradecamethyl-	17.878	2.80
24	Heptasiloxane tetradecamethyl-	19.153	2.46
25	Heptasiloxane tetradecamethyl-	20.324	1.85
	Total		100

Table 7: Phytocomponents identified in Adansonia digitata ethanolic extract.

Peak#	Name	R.Time	Area%
1	2-Thiophenecarboxylic acid hydrazide	2.849	1.35
2	Cyclohexanecarboxylic acid methyl ester	4.374	25.66
3	Cyclohexanecarboxylic acid ethyl ester	5.083	6.38
4	2- Butenedioic acid(z)-dimethyl ester	5.211	13.13
5	Sucrose	7.352	4.22
6	1-Dodecanol -	7.783	2.72
7	Pentanoic acid ,4-oxo,ethyl ester	8.528	3.09
8	Diethylbis(hydoxymethyl)malonate	8.939	2.12
9	Benzene,(1-propylnonyl)-	9.310	1.26
10	1-Hexadecanol	9.532	2.51
11	Ethanol ,2-(dodecyloxy)-	9.935	2.72
12	Heptasiloxane hexadecamethyl	12.426	1.15
13	1-Heptanamine,N,N-dimethyl	12.688	4.62
14	Hexasiloxane,tetradecamethyl-	13.888	1.74
15	2H-Indol-2-one,1-(2,6-dichlorophenyl)-1,3-di	14.722	2.27
16	Heptasiloxane hexadecamethyl-	15.236	2.03
17	9-Octadecenamide,(z)-	15.176	2.35
18	Heptasiloxane hexadecamethyl-	16.506	3.15
19	Heptasiloxane hexadecamethyl-	17.878	4.32
20	Heptasiloxane hexadecamethyl-	19.154	4.85
21	Heptasiloxane hexadecamethyl-	20.325	4.81
22	Heptasiloxane hexadecamethyl-	21.592	3.57
	Total		100

Table 8: Phytocomponents identified in Hibiscus sabdariffa ethanolic extract.

Peak#	Name	R.Time	Area%
1	Glyceraldehyde	4.473	1.06
2	Benezeethanol,4-hydroxy-	7.393	9.92
3	Alpha-Methyl mannofuranoside	9.573	80.05
4	Heptasiloxane hexadecamethyl-	16.509	1.82
5	Heptasiloxane hexadecamethyl-	17.881	2.47
6	Hexasiloxane,hexadecamethyl-	19.157	2.56
7	Heptasiloxane hexadecamethyl-	20.326	2.12
	Total		100

Table 9: Phytocomponents identified in Tamarindus indica ethanolic extract.

Peak#	Name	R.Time	Area%
1	Glycerin	2.869	0.57
2	Glyceraldehyde	4.533	3.50
3	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	4.681	3.39
4	Methanamine,1,1-bis(2-2-dimethyl propoxy)-N	5.206	1.50
5	5-Hydroxymethylfurfural	5.485	30.39
6	1,2,3-Propanetriol,1-acetate	5.614	2.67
7	Cyclohexxne,1-ethyl-4-methyl-,trans-	5.689	4.25
8	2-Propanol,1-propoxy-	5.858	3.22
9	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6	5.941	4.17
10	Maltol	6.208	1.23
11	1-[N-Methylpiperazine] ethanol	6.388	1.53
12	Butanoic acid,3-hydroxy-3methyl-	6.549	1.05
13	2-Furanmethanol,tetrahydro-,acetate	6.947	3.93
14	Sucrose	7.609	20.62
15	1-Dodecanol	7.783	0.97
16	beta-D-Glucopyranose,-O-beta-D-galactor	7.942	2.17
17	Glucitol,6-O-nonyl-	8.764	8.73
18	Tetrahydro-4H-pyran-4-ol	8.958	5.59
	Total		100

DISCUSSION

The present investigation provides a comprehensive comparative evaluation of the phytochemical architecture and antioxidant capacity of five ethnobotanically significant Sudanese fruit species. The empirical data collectively demonstrate substantial interspecific variability in extraction efficiency, secondary metabolite profiles, phenolic content, and free radical scavenging proficiency, thereby underscoring the chemotaxonomic diversity inherent to these indigenous botanical resources.

Extraction Yield Variability: The gravimetric extraction yields exhibited a pronounced hierarchy, with T. indica (71.0%) substantially outperforming all other species. This exceptionally high recovery is plausibly attributable to the elevated concentration of low-molecular-weight carbohydrates, organic acids (principally tartaric acid, citric acid, and malic acid), and highly hydrophilic pectic polysaccharides intrinsic to tamarind pulp, which demonstrate exceptional solubility in the aqueous-ethanolic extraction medium.[15] Comparable extraction efficiencies for tamarind have been documented by Sudjaroen et al.[16], who reported yields exceeding 65% with hydroalcoholic solvent systems. Conversely, the comparatively modest yields recorded for C. siliqua (29.0%) and G. tenax (29.56%) likely reflect a higher lignocellulosic matrix composition and the presence of lipophilic cuticular barriers that impede exhaustive solvent penetration. Kyriacou et al.[17] similarly reported extraction yields ranging from 28% to 32% for carob pods across various maturity stages, attributing the lower recoveries to the recalcitrant nature of the seed-containing pericarp.

Phytochemical Diversity and Chemotaxonomic Significance: Qualitative phytochemical profiling revealed a ubiquitous distribution of flavonoids, coumarins, triterpenoids, and anthraquinones across all five taxa, while alkaloids and steroidal nuclei were consistently undetectable. This pattern of secondary metabolite accumulation corroborates the findings of Ndiaye et al.[18], who reported analogous phytochemical fingerprints for West African specimens of A. digitata. The consistent absence of alkaloids is particularly noteworthy, as it suggests that the traditional therapeutic applications of these species—encompassing gastrointestinal regulation, cardiovascular support, and metabolic modulation—are unlikely to involve alkaloid-mediated mechanisms. The exclusive detection of saponins in G. tenax, T. indica, and A. digitata—and their absence from C. siliqua and H. sabdariffa—is significant, as these amphiphilic glycosides are recognized for their immunomodulatory, hypocholesterolemic, and membrane-permeabilizing properties.[19] The exceptional tannin content quantified in H. sabdariffa (113,542.16 µg CAE/g) provides empirical validation for its traditional employment as a natural astringent in the management of diarrheal conditions, as previously documented by Puro et al.[20] This remarkable tannin concentration—approximately tenfold higher than that observed in other species—is consistent with the findings of Fullerton et al.[21], who reported potent antibacterial activity of roselle extracts against enteric pathogens, mediated in part through tannin-induced protein precipitation and membrane disruption.

Phenolic and Flavonoid Quantification: The quantitative determination of phenolic and flavonoid pools established a distinct hierarchy, with C. siliqua exhibiting the uppermost concentrations of both TPC (1149.37 µg GAE/g) and TFC (561.36 µg CAE/g). These values are remarkably congruent with those reported for Mediterranean carob ecotypes by Macho-Gonzalez et al.[22], who documented TPC ranging from 850 to 1250 µg GAE/g depending on genotype and geographical provenance. The exceptionally elevated TPC observed for A. digitata (1096.26 µg GAE/g) corroborates the findings of Braca et al.[7], who characterized Malian baobab fruit pulp as a premier source of phenolic nutrients, attributing the high phenolic load to the presence of procyanidins, flavonoid glycosides, and hydroxycinnamic acid derivatives. The comparatively attenuated TPC and TFC values recorded for T. indica (382.26 µg GAE/g and 87.75 µg CAE/g, respectively) are nonetheless consistent with the findings of Bhadoriya et al.[23], who demonstrated that tamarind's antioxidant potential is predominantly mediated by non-phenolic reducing agents—including ascorbic acid, tocopherols, and beta-carotene—rather than by phenolic secondary metabolites alone. This observation underscores the importance of comprehensive phytochemical characterization that extends beyond phenolic quantification alone.

Correlation between Phenolic Content and Antioxidant Capacity: A salient observation of this investigation is the robust positive correlation between TPC/TFC and DPPH radical scavenging efficacy. C. siliqua, with the highest phenolic and flavonoid loads, correspondingly demonstrated the most potent RSA (80.0%), a finding that aligns with the mechanistic understanding that phenolic hydroxyl groups function as hydrogen atom donors, effectively terminating oxidative chain reactions.[8] The remarkable RSA exhibited by H. sabdariffa (78.0%) and A. digitata (76.0%) further substantiates their established status as potent radical scavengers. Ismail et al.[24] similarly reported DPPH RSA values exceeding 75% for optimized roselle extracts, attributing the activity to the synergistic interplay between anthocyanins (delphinidin-3-sambubioside and cyaniding-3-sambubioside) and copigmenting phenolic acids. For baobab, Tembo et al.[25] documented comparable radical neutralizing capacity, emphasizing that thermal processing and storage conditions significantly influence the retention of antioxidant activity. The comparatively moderate RSA observed for T. indica (59.0%) and G. tenax (57.0%) does not diminish their therapeutic relevance; rather, it suggests that their primary antioxidant mechanisms may involve

metal chelation or enzymatic modulation rather than direct radical neutralization, as proposed by Elhassan and Yagi[5] for Grewia species.

GC-MS Metabolomic Signatures: The GC-MS chromatographic analysis provided definitive chemoprofiles that both confirm and expand upon prior phytochemical knowledge. The predominance of alpha-methyl mannofuranoside in C. siliqua (51.26%) and H. sabdariffa (80.05%) represents a noteworthy finding. Papagiannopoulos et al.[26] identified this furanoside derivative as a characteristic component of carob pod extracts, suggesting its potential involvement in cellular membrane stabilization and immunomodulation. The identification of the 2H-indol-2-one derivative (28.02%) in C. siliqua is particularly significant; indole alkaloids and their derivatives are recognized for their diverse pharmacological activities, including neuroprotection and anti-inflammatory modulation.[27] In T. indica, the high abundance of 5-hydroxymethylfurfural (5-HMF) at 30.39% warrants careful interpretation. While 5-HMF is classically considered a Maillard reaction artifact generated during thermal processing, recent pharmacological investigations have demonstrated its concentration-dependent antioxidant and cytoprotective properties, acting as a precursor to free radical scavenging metabolites.[28] The dominance of sucrose in G. tenax (19.15%) and T. indica (20.62%) authentically validates their traditional employment as natural sweetening and energy-restorative agents in the Sudanese diet. Additionally, the presence of cyclohexanecarboxylic acid methyl ester (25.66%) and 2-butenedioic acid dimethyl ester (13.13%) in A. digitata suggests a distinctive ester-rich metabolic pool; Braca et al.[7] preliminarily indicated that such organic acid esters may contribute to baobab's anti-inflammatory efficacy, warranting further investigation through targeted bioassay-guided fractionation.

Implications for Nutraceutical Application: The cumulative findings of this investigation carry substantial implications for the valorization of these underutilized Sudanese botanical resources. The demonstrated antioxidant capacity, coupled with the favorable phytochemical profiles, positions these fruit extracts as viable natural alternatives to synthetic antioxidants (such as butylated hydroxytoluene and butylated hydroxyanisole) in food preservation systems. Moreover, the chemotaxonomic distinctiveness established through GC-MS metabolomic profiling provides the foundation for developing standardized quality control protocols—essential prerequisites for commercializing botanical products intended for dietary supplementation. The superior performance of C. siliqua as an antioxidant repository, corroborated by its substantial phenolic and flavonoid loads, designates this species as the most promising candidate for nutraceutical development among the five investigated taxa.

CONCLUSION

In synthesis, this investigation delivers a systematic comparative appraisal of the phytochemical architecture and oxidative stress-mitigating capacity of five indigenous Sudanese fruit species—Ceratonia siliqua, Grewia tenax, Adansonia digitata, Hibiscus sabdariffa, and Tamarindus indica. The empirical evidence unequivocally establishes C. siliqua as the most prolific repository of phenolic bioactives, manifesting the highest TPC (1149.37 µg GAE/g), TFC (561.36 µg CAE/g), and DPPH radical scavenging activity (80.0% RSA), closely followed by H. sabdariffa (78.0%) and A. digitata (76.0%). The GC-MS-derived phytochemical signatures not only authenticate the ethnopharmacological claims associated with these plants but also unveil distinctive chemical markers—namely alpha-methyl mannofuranoside, 5-hydroxymethylfurfural, and specific organic acid esters—that are instrumental for future quality assurance protocols and metabolomic standardization. Collectively, these findings strongly advocate for the strategic valorization of these underutilized Sudanese botanical resources as cost-effective, naturally derived functional food ingredients and dietary supplements with significant health-promoting potential. The study further underscores the importance of integrating traditional ethnobotanical knowledge with modern analytical methodologies to facilitate the evidence-based development of phytopharmaceuticals, thereby bridging the critical gap between historical wisdom and contemporary nutritional science.

Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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