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Bioactive Compounds Investigation and Antioxidant Activities in Extracts and Fractions of *Tamarindus Indica* (Aradib) From Sudan

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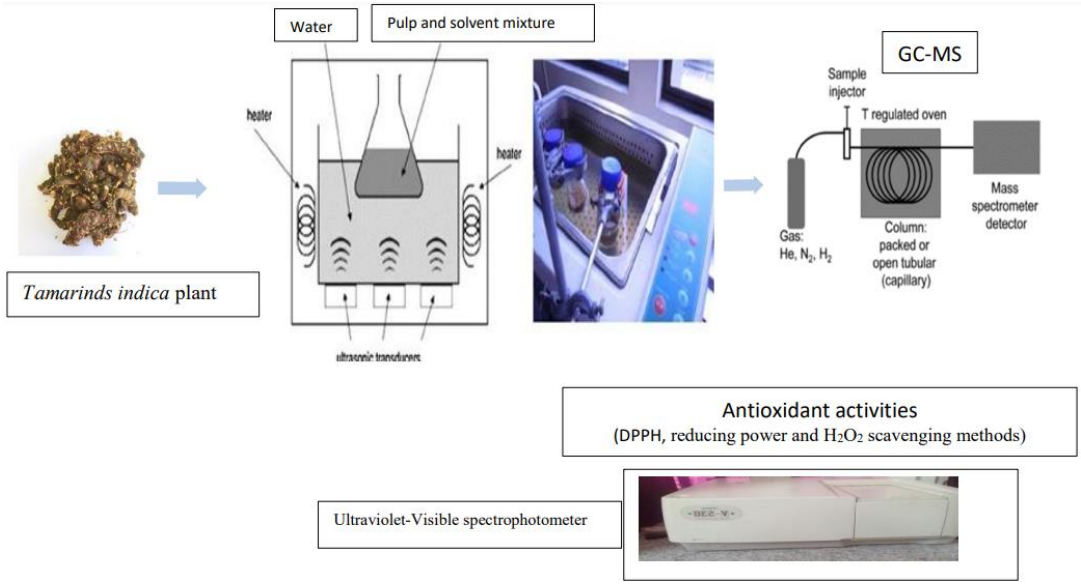
GRAPHICAL ABSTRACT



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ABSTRACT

Tamarindus indica is commonly used in Sudan as an edible plant and for medicinal use. This study aimed to determine bioactive compounds and antioxidant activities in extracts and fractions of *Tamarindus indica* pulp grown in Sudan. The powdered plant was extracted with ethanol, ethyl acetate and acetone using ultrasonic. The residue of the ethanol extract was fractionated using successive solvents. The extracts and fractions were determined for their phenolics, flavonoids and tannins contents. They analyzed bioactive compounds by Gas Chromatography-Mass Spectrophotometer and also evaluated their antioxidant activities using the 2, 2-diphenyl-1-picryl-hydrazyl, reducing power ability assay and hydrogen peroxide scavenging methods. The results show that the total phenolics, flavonoids and total tannins levels were significantly higher in the ethyl acetate fraction than in other extracts and fractions. GC-MS analyses of extracts and fractions showed a variety of bioactive compounds such as Hexadecanoic acid, Stigmasterol, Maltol, Sitosterol, Vitamin E, Allose, Tartaric acid, and Vaccenic acid. All the extracts and fractions exhibited

good antioxidant activities. The extracts and fractions of *Tamarindus indica* pulp are sources of natural antioxidants and other bioactive compounds, which benefit human health and the possibility of application in the pharmaceutical industry.

1. INTRODUCTION

Tamarindus indica is known in the Sudanese communities as Aradib. This plant has great interest and has been reported to have good nutritional value and medicinal properties. *Tamarindus indica L*. (Tamarind), family Leguminosae, is a widely used medicinal plant. It is found in virtually all tropical climatic regions, from India through Africa to the Caribbean and South America and up to Southern Florida [1]. Tamarind is indigenous to tropical Africa, particularly in Sudan, and it is Sudan's most famous plant product [2]. The tamarind tree produces edible, pod-like fruit, used extensively in cuisines worldwide. They are used as food ingredients and in traditional treatments of various diseases [3]. *Tamarindus indica* is a plant used traditionally for wound healing, snake bites, abdominal pain, colds, inflammations, diarrhoea, diarrhoea, helminth infections, and fever [4]. It is also used as a flavouring agent to impart flavour to various dishes and beverages [5].

Tamarindus indica possesses the amount of vitamins B and C, carbohydrates (glucose, fructose and arabinose as inverted sugars), fat, proteins, acids, and minerals (Copper, Iron, Cadmium, Zinc, Lead, Sodium, Potassium, Calcium, Magnesium and Phosphorus) [2, 5]. The plant shows various types of activities such as antidiabetic, hypolipidemic, antioxidant, hepatoprotective, antimicrobial, anti-snake venom analgesic, anti-inflammatory, cytotoxic and antiproliferative activities [5-7]. *Tamarindus indica* pulp has the highest concentration of tartaric acid (23.75 mg/g ± 0.0.36) method than other plants collected from Sudan, such as *Adansonia* digitate (0.574± 0.062), *Hyphaene thebaica* (0.952± 0.052) and *Hibiscus sabdariffa* (8.43± 0.008) [8].

The pulp of *Tamarindus indica* is rich in biological activities and possesses an antioxidant property that makes it suitable for cosmetic applications. The potential use of tamarind pulp extract in cosmetic formulations, especially in antiaging products [9], is a promising area of research that can inspire new developments in the field. The use of the ultrasonic extraction method for the identification and determination of bioactive compounds in this plant has not been reported before. In addition, treatment on the residue of extract such as fractionation steps provides the ability of fractions to scavenge free radicals which is responsible for some diseases, which have not been studied previously for this plant. Therefore, it is required to determine total phenolics, flavonoids, and tannins and measure antioxidant activities in extracts and fractions of *Tamarindus indica* pulp obtained by ultrasonic extraction method, in addition to analysis of bioactive compounds of extracts and fractions by GC-MS.

2. EXPERIMENTAL METHODS

2.1. Plant material and Samples preparation

Tamarindus indica fruits were collected from Khartoum states, Sudan. The plant material was identified and authenticated by the herbarium unit of the National Center for Research, Khartoum state, Sudan. The pulp of *Tamarindus indica* was removed from the seeds. The samples were cleaned and allowed to dry in the dark at 25 ºC for two weeks. The dried samples were ground to a fine powder using a food grinder, sieved through a 0.08 mm sieve, then kept in a labelled glass jar and stored in the refrigerator.

2.2. Chemicals and reagents

Ethanol (99.8%), Acetone (99.7%), Ethyl acetate (99.0%), Chloroform, Petroleum ether, Lead acetate (AR), Sodium nitrite, Sodium carbonate, Aluminum chloride, Sodium hydroxide, Potassium ferric cyanide, Ferric chloride, and Tannic acid (AR) were obtained from s. d fine- CHIME Limited -India. Gallic acid (%), Folin-Ciocalteu reagent (phenol reagent), Dimethyl sulfoxide (DMSO), 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Quercetin (%) were obtained from Sigma Chemical Co.

(USA). All other reagents and chemicals were of analytical grade, and double-distilled water was used throughout the study.

2.3. Extraction and fractionation

The extraction of phytochemicals from the *Tamarindus indica* pulp was carried out according to the [10] method with some modifications. The extraction was done using an ultrasonic technique (BANDELIN electronic- Germany) using three solvents of increasing polarity: ethyl acetate, acetone, and ethanol. Two grams of powdered samples were weighed and placed in the glass beaker, and then 20 ml of the solvent was added to each beaker (the ratio of sample to solvent was 1g /10 ml w/v). The extracts were filtered through filter paper. The extracts were dried at 25 ºC for 48h; the residue of the ethanol extract was used in fractionation steps. The residue of ethanol was fractionated by petroleum ether and chloroform, and equal amounts of chloroform fraction were separated into two groups (the ratio of the residue of extract to solvent was 1:1). The first group was fractionated using ethyl acetate; the second group was fractionated by acetone solvent. The ethyl acetate and acetone fractions were collected individually and dried at 25 ºC. All extracts and fractions were stored in a clean container and were kept in a freezer for analysis.

2.4. Determination of phytochemicals

In this study, the quantification of the contents of phenolic compounds, total flavonoids, and total tannins present in the plant extracts and fractions studied was carried out using a UV-visible spectrophotometer (V-530, JASCO, Japan).

2.4.1. Determination of total phenolic compounds

With some modification, the total phenolic compounds were measured by spectrophotometry using Folin–Ciocalteu reagent [11]. One mg of each sample was dissolved in 1000 µl of ethanol (1mg/ml) in different test tubes, and the volume was made up to 3000µl with distilled water. Subsequently, 2500µl of Folin–Ciocalteu reagent 10% 54 (v/v) was added to each sample. After 5 min, 2500µl of sodium carbonate (7.5% w/v) was added to the mixture; the test tubes were kept in the dark for 2 hours at room temperature. The blue colour was developed in each tube, and the colour's intensity is directly proportional to the phenolic compounds. After incubation, the absorbance of samples was measured at 765 nm against blank using an Ultraviolet-Visible spectrophotometer, using gallic acid as the standard. Results were expressed as gallic acid equivalents mg QAE/g; all samples were analysed in triplicates.

2.4.2. Determination of total flavonoids

The total flavonoids of the samples were determined using aluminium chloride by an Ultraviolet-Visible spectrophotometer [12], with some modifications. One mg of each sample was dissolved in 1000 µl of ethanol (1mg/ml) in different test tubes, 1000 µl of each sample was added to each test tube, and the volume was made up to 2000 µl with distilled water, 300 µl of sodium nitrite solution (5% w/v) was added to the mixture, shaken well and incubated for 5 min at room temperature, then 300 µl of aluminium chloride solution (10% w/v) was added to each tube, shaken, and left to stand for 5min, then 2000 µl of sodium hydroxide (1 M) was added to the mixture, shaken and left to stand for 15 min. Absorbance was measured at 415 nm against a blank using an Ultraviolet-Visible spectrophotometer, using quercetin (100-800 mg/l) as standard and total flavonoids (mg QE/g) were expressed as quercetin equivalents; all samples were analysed in triplicates.

2.4.3. Determination of total tannins

Total tannins in samples were determined by UV-visible spectrophotometer using potassium ferric cyanide and ferric chloride reagents [13]. One mg of each sample was dissolved in 1000 µl of ethanol (1 mg/ml) in different test tubes, 1000µl of each sample (1 mg/ml) was transferred to each test tube, then 1000 µl of potassium ferric cyanide (1% w/v) and 1000 µl of ferric chloride $FeCl_3$ (1% w/v) were added, and the volume was made up to 10000 µl with distilled water. The reaction mixture was shaken and left to stand for 5 min, and a green colour was developed. Absorbance was measured

at 510 nm against a blank using an Ultraviolet-Visible spectrophotometer. Total tannins in samples were expressed as tannic acid equivalents (mg TAE/g) using tannic acid as standard, and all samples were analysed in triplicates.

2.5. Identification of phytochemicals in extracts and fractions by Gas chromatography-Mass spectrometer (GC-MS)

All extracts and fractions of *Tamarindus indica* pulp were analyzed by Gas chromatography-mass spectrometer to identify phytochemicals [14]. GC-MS Conditions: identifications analysis of the samples was carried out using the GC-MS model (GC-MS-QP2010-Ultra), japan Shimadzu Company, serial number 020525101565SA, and capillary column Rtx-5ms (30m×0.25mm diameter×0.25µm thickness) coupled to mass spectrometer detector. Ultra-high purity helium (99.99%- Germany) as the carrier gas passed with a constant flow rate of 1.61 ml/min, the volume of injection volume was 1µl, and the sample was injected using split mode.

The temperature program was meticulously designed, starting from 60 ºC with a rate of 10℃/min, reaching a final temperature of 300 ºC with a 5-minute hold time. The injection port temperature was 300 ºC, the ion source temperature was 200 ºC, and the interface temperature was 250 ºC. The mass spectrometer detector was operated in electron impact ionization mode with an ionizing energy of 70 Ev. The sample was analyzed using scan mode in the range from m/z of 40-600 of charges to ratio, and the total run time was 29 minutes. The percentage composition of the extract constituents was expressed as a percentage by peak area. The identification of components for the sample was achieved by comparing their retention time and mass fragmentation pattern with those available in the National Institute of Standards and Technology (NIST) records library.

2.6. Determination of antioxidant activities in extracts and fractions

2.6.1. DPPH free radical scavenging assay

The antioxidant activity of extracts and fractions was determined by DPPH free radical scavenging [15]. One mg of each sample was dissolved in1000 µl of ethanol (1 mg/ml) in different test tubes. 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) was prepared in ethanol (300 µM). 1000 µl of DPPH solution was added to 1000 µl of sample solution in different test tubes; the test samples were allowed to react with 2, 2 diphenyl-1-picryl-hydrazyl stable free radical (DPPH) for 30 min at room temperature. After incubation, the absorbance was measured at 517 nm using an Ultraviolet-Visible spectrophotometer. A solution of 100 µl absolute ethanol and1000 µl DPPH was used as a control. The reference standard was propyl gallate. All tests and analyses were run in triplicate. The radical scavenging activity was calculated as follows:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Absorbance (control) - Absorbance (sample)}}{\text{Absorbance (control)}} \times 100\%$$

2.6.2. Reducing power assay

The antioxidant activity of extracts and fractions was determined by reducing power [16] with some modification. One mg from each sample was dissolved in 1000 µl of ethanol (1mg/ml) in a different test tube and was mixed with 2000 µl of phosphate buffer solution (0.2 M, pH 6.6), then 2000 µl potassium ferricyanide (1% w/v) was added to each extract. The mixture was incubated at 50 °C for 20 min. Then, 2000µl of trichloroacetic acid (10% w/v) was added to the mixture, followed by centrifugation at 3000 rpm for 10 min at room temperature. 2000 µl of the supernatant (Above layer) was taken and mixed with 2000 µl of distilled water. Then 500 µl ferric chloride (0.1% w/v) was added. The reaction mixture was incubated for 30 minutes at 25 ºC until a green colour was formed. Absorbance was read at 700 nm against blanks that contained all reagents except sample extracts using an ultraviolet-visible spectrophotometer. Ascorbic acid was used as standard. All tests and analyses were run in triplicate. The higher absorbance indicates greater reducing capacity, which was calculated as follows:

$$\text{Reducing power (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\ \%$$

2.6.3. Hydrogen peroxide scavenging assay

Hydrogen peroxide radical scavenging of extracts and fractions was determined according to Serhat Keser et al. [17]. One mg from each sample was dissolved in 1000 µl of ethanol (1 mg/ml) in each test tube. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (0.2M, pH= 7.4). In different test tubes, 1000 µl of each sample (1 mg/ml) was added to hydrogen peroxide solution (600 µl, 40 mM). The solution was incubated at room temperature for 10 minutes. The absorbance of the reaction mixture was read at 230 nm using an Ultraviolet-Visible spectrophotometer. Ascorbic acid was used as a standard and blank solution containing phosphate buffer without hydrogen peroxide. The phosphate buffer with hydrogen peroxide was used as a control. All tests and analyses were run in triplicate. The percentage of H_2O_2 scavenged was calculated using the following formula:

$$H_2O_2 \text{ scavenging radical activity (\%)} = \frac{\text{Absorbance (control) - Absorbance (sample)}}{\text{Absorbance (control)}} \times 100\%$$

3. RESULTS AND DISCUSSIONS

3.1. Phytochemicals in extracts and fractions of *Tamarindus indica* pulp

The results of phytochemicals (total phenolics, total flavonoids, and total tannins) are presented in Table 1. From the result, the ethyl acetate fraction gave the highest total phenolic compounds (27.08 mg/g), total flavonoids (16.56 mg/g), and total tannins (7.54 mg/g) than other extracts and fractions. Total phenolics and total flavonoids of *Tamarindus indica* was higher content than that reported by *A. Lamien-Meda et al.* (2008) [11] (957.33 mg/100g- 888.67 mg/100g) and (2.18mg/100g- 5.68 mg/100g) respectively.

In this research, the fractional extraction method has proven to be a suitable tool for determining the phytochemical content of *Tamarindus indica*. The use of ethyl acetate and acetone solvents in this method has been crucial in revealing the antioxidant activity of these phytochemicals.

TABLE I. Total phenolics, total flavonoids, and total tannins in *Tamarindus indica* extracts and fractions (mg/g)*.

	Ethanol extract	Ethyl acetate extract	Acetone extract	Ethyl acetate fraction	Acetone fraction
Total phenolics	24.53±0.4	23.42±0.3	23.98±0.4	27.08±0.4	18.53±0.4
Total flavonoids	11.13± 0.1	13.12± 0.4	7.84± 0.7	16.56± 0.08	2.11± 0.8
Total tannins	3.35± 0.4	3.35± 0.4	2.21± 0.6	7.54± 0.09	5.69± 0.3

* Values as: Mean ± Standard Deviation SD (n=3).

3.2. Bioactive compounds of extracts and fractions identified by Gas chromatography-Mass spectrometer (GC-MS)

Bioactive compounds were identified in extracts and fractions of Tamarindus, indicating GC-MS analysis. Their retention time (Rt) and percentage of area (Area %) are shown in Table 2. The National Institute of Standards and Technology (NIST) database was used to interpret GC-MS mass spectra.

The ethanol extract, for instance, contained five phytochemicals as antioxidants (18) which were: Maltol, Tartaric acid, Allose, n- Hexadecanoic acid and Hexadecanoic acid ethyl ester at retention times 4.192, 8.854, 11.52, 15.726 and 15.964 minutes respectively. These compounds, along with γ- sitosterol, which was also found in the extract, have potential applications as hypolipidemic agents, demonstrating the practical implications of our research.

Ethyl acetate extract contained two phytochemicals with antioxidant properties, which were Hexadecanoic acid methyl ester and 2,2-methylenebis[6-(1,1-dimethyl ethyl)]-4-methyl phenol detected at retention times 15.317 and 19.92 minutes respectively. Maltol, Allose, n-hexadecanoic acid and 4-oxo-pentanoic acid were detected in acetone extract at retention times of 4.199, 11.231, 15.744 and 4.762 minutes, respectively. However, the acetone fraction contained two

phytochemicals with antioxidant properties were detected which were, Allose and n-hexadecanoic acid, at retention times of 11.071 and 15.731 minutes, respectively, and also detected 4-oxo-pentanoic acid at a retention time of 4.749 minutes. Five phytochemicals as antioxidants were detected in ethyl acetate fraction, which was vitamin E, Stigmasterol, 2,4-bis(1,1-dimethyl ethyl)phenol, Hexadecanoic acid methyl ester, and Hexadecanoic acid ethyl ester at retention times 25.122, 26.431, 10.888, 15.312 and 15.973 minutes respectively, and also detected Oleic acid at retention time 17.604 minutes.

TABLE II. Bioactive compounds in extracts and fractions of *Tamarindus indica* by GC-MS.

No.	Name	Rt	Area%
	Ethanol extract		
1	5-Methyl- 2-furancarboxaldehyde	3.666	0.69
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.809	0.20
3	1-(2-thienyl)-1-Pentanone	3.872	0.08
4	N-Methyl-2-Propenamide	4.136	0.26
5	Maltol	4.192	0.28
6	Dihydro-3-methyl- 2,5-Furandione	4.446	0.60
7	Benzeneacetaldehyde	4.664	0.09
8	4-Oxo-pentanoic acid	4.755	0.46
9	Acetic acid butyl ester	5.205	0.25
10	Methyl 2-furoate	5.234	1.55
11	Levoglucosenone	5.630	0.88
12	2,2-Dimethyl-3-heptanone	5.810	0.09
13	2,3-Dihydro-3,5-dihydroxy-6-methyl- 4H-Pyran-4-one	6.111	3.41
14	2-Pentanol propanoate	6.830	0.43
15	1,4,3,6-Dianhydro-α-d-glucopyranose	7.080	1.87
16	5-Hydroxymethylfurfural	7.498	24.36
17	3-Heptanol	8.509	0.86
18	DL-Tartaric acid	8.854	0.30
19	D-Allose	11.52	12.82
20	1,6-Anhydro-α-d-galactofuranose	12.717	4.56
21	4-O-Methylmannose	14.721	44.49
22	n-Hexadecanoic acid	15.726	0.36
23	Hexadecanoic acid ethyl ester	15.964	0.08
24	9-Oxabicyclo[6.1.0]non-6-en-2-one	16.238	0.26
25	1-(1-propenyl)- pyrrolidine,	16.387	0.05
26	8-Azabicyclo[3.2.1]octane-8-carboxaldehyde	16.446	0.02
27	9-Octadecenoic acid methyl ester	16.997	0.07
28	Methyl stearate	17.215	0.04
29	Cis- vaccenic acid	17.402	0.13
30	(E)-9-Octadecenoic acid ethyl ester	17.595	0.14
31	13-Docosenoic acid methyl ester	20.414	0.01
32	γ - Sitosterol	27.016	0.31
	Ethyl acetate extract		
1	5-Hydroxymethylfurfural	7.938	8.43
2	1-Tetradecene	9.219	1.28
3	Tetradecane	9.311	0.37
4	4-O-Methylmannose	13.085	44.12
5	Hexadecanoic acid methyl ester	15.317	1.35
6	2,2'-Methylenebis[4-methyl- 6-(1,1-dimethylethyl)- phenol	19.920	1.28
7	14-Methyl-8-hexadecenal	20.249	5.00
8	γ -Sitosterol	27.061	38.17
	Acetone extract		
1	5-Methyl-2-furancarboxaldehyde	3.673	0.56

2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.815	0.35
3	2H-pyran-2,6(3H)-dione	4.152	0.17
4	Maltol	4.199	0.07
5	Dihydro-3-methyl-2,5-furandione	4.470	0.30
6	Benzeneacetaldehyde	4.675	0.07
7	4-Oxo-pentanoic acid	4.762	0.52
8	Methyl 2-furoate	5.251	0.78
9	Levoglucosenone	5.647	0.24
10	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	6.125	3.45
11	2-Pentanol propanoate	6.847	0.41
12	1,4,3,6-Dianhydro-α.-d-glucopyranose	7.090	1.21
13	5-Hydroxymethylfurfural	7.475	27.40
14	D-Allose	11.231	10.02
15	1,6-Anhydro-β-glucofuranose	12.572	5.56
16	3-O-Methyl-d-glucose	14.398	48.56
17	n-Hexadecanoic acid	15.744	0.33
	Ethyl acetate fraction		
1	1-Dodecene	6.522	0.75
2	2-Methoxy-4-vinylphenol	8.481	0.83
3	3-Hexadecene	9.213	1.41
4	Tetradecane	9.307	0.74
5	N,N-Dimethyl-1-pentadecanamine	10.674	0.63
6	2,4-Bis(1,1-dimethylethyl)- phenol	10.888	0.29
7	1-Pentadecene	11.683	1.49
8	Hexadecane	11.761	0.66
9	N,N-Dimethyl-1-nonadecanamine	12.988	1.09
10	1-Heptadecene	13.915	1.08
11	Hexadecanoic acid methyl ester	15.312	1.25
12	1-Heneicosanol	15.944	0.69
13	Hexadecanoic acid ethyl ester	15.973	2.58
14	1-Hexadecanol	16.859	1.22
15	9,12-Octadecadienoic acid methyl ester	16.960	3.37
16	9-Octadecenoic acid methyl ester	17.005	2.20
17	Methyl stearate	17.222	1.06
18	Linoleic acid ethyl ester	17.564	9.84
19	Oleic Acid	17.604	0.79
20	1,2-15,16-Diepoxyhexadecane	20.220	2.90
21	Hexadecanoic acid trimethylsilyl ester	20.396	3.47
22	Stigmast-5-en-3-ol oleate	24.951	3.60
23	Tocopherol	25.122	5.85
24	Stigmasterol	26.431	8.77
25	γ - Sitosterol	27.040	27.14
26	Furcosterol	27.232	7.53
27	17.β.-Methyl-18-nor-17-isopregna-4,13-dien-16.β-ol-3,20-dione	28.720	8.77
	Acetone fraction		
1	5-Methyl-2-furancarboxaldehyde	3.676	0.55
2	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	3.808	0.62
3	Dihydro-3-methyl- 2,5-Furandione	4.455	0.29
4	Benzeneacetaldehyde	4.672	0.08
5	4-Oxo-pentanoic acid	4.749	0.79
6	Methyl 2-furoate	5.250	0.34
7	3-Methyl-4-methylene- 2-hexanone	5.295	0.64
8	Levoglucosenone	5.649	0.07
9	Acetic acid heptyl ester	5.682	0.06

10	2,2-Dimethylpropanoic anhydride	5.816	0.13
11	2,3-Dihydro-3,5-dihydroxy-6-methyl- 4H-pyran-4-one	6.107	4.24
12	2-Pentanol propanoate	6.829	0.22
13	1,4,3,6-Dianhydro-α-d-glucopyranose	7.079	0.20
14	5-Hydroxymethylfurfural	7.439	30.98
15	D-Allose	11.071	4.88
16	1,6-Anhydro-β-D-glucofuranose	12.440	3.02
17	4-O-Methylmannose	14.305	52.45
18	n-Hexadecanoic acid	15.731	0.35
19	3.β-Cholest-5-en-3-ol, carbonochloridate	24.950	0.09

3.3. Antioxidant Activities of *Tamerindus indica* extracts and fractions

Antioxidant activities of extracts and fractions were measured using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), reducing power (RP) and hydrogen peroxide scavenging (H_2O_2); the results are shown in Table 3. from the results all extracts and fractions have antioxidant activities. The highest percentage of DPPH of Tamerindus indica was 84.00% observed in acetone extract. The reducing power of Tamarindus indica varied from 95.97% for acetone fraction to 55.51% for ethanol extract. The percentage of hydrogen peroxide scavenging varied from 88.18% for acetone fraction to 68.82 % for ethyl acetate extract. DPPH activity of *Tamerindus indica* pulp differed from that reported by S. Farooq et al. (2022) [15].

Extracts and fractions of plants studied can be used as accessible sources of natural antioxidants. The antioxidant activities of Tamarindus indica have been attributed to Maltol, tartaric acid, Allose, N-Hexadecanoic acid, Hexadecanoic acid, vitamin E, Stigmasterol, and 2,4-bis(dimethyl ethyl)phenol. Thus, the antioxidant activities of this plant are due to the presence of several phytochemicals, mainly antioxidant compounds.

TABLE III. Antioxidant activities in extracts and fractions of *Tamarindus indica.*

Extracts and Fractions	RSA%± SD*		
	DPPH	RP	H_2O_2
Ethanol extract	64.6± 0.24	55.51± 0.32	75.52± 0.22
Ethyl acetate extract	76.29± 0.05	76.79± 0.01	68.82± 0.01
Acetone extract	84.0± 0.09	85.93± 0.03	75.69± 0.03
Ethyl acetate fraction	63.43± 0.15	71.79± 0.00	88.62± 0.42
Acetone fraction	63.70± 0.01	95.97± 0.01	75.18± 0.01

* RSA%± SD: Radical Scavenging Activity ± standard deviation.

4. CONCLUSIONS

Tamarindus indica pulp has an attractive commercial future for producing drinks, sweets, and jams on an industrial scale in Sudan. This study shows a high variation of phytochemicals and other bioactive compounds in extracts and fractions of *Tamarindus indica* pulp, which may act as natural antioxidants. The phytochemicals and bioactive compounds could contribute significantly to protecting against several diseases. The fractional extraction method is suitable for determining the total phenolic compounds, flavonoids and total tannins of *Tamarindus indica* pulp using ethyl acetate solvent. Further fractionation of active extracts is recommended to determine phytochemicals and bioactive compounds responsible for antioxidant properties.

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References

[1] U. U. Nwodo, G. E. Obiiyeke, V. N. Chigor, and A. I. Okoh, “Assessment of tamarindus indica extracts for antibacterial activity,” *Int. J. Mol. Sci.*, vol. 12, no. 10, pp. 6385–6396, 2011, doi: 10.3390/ijms12106385.

[2] A. Moneim, E. Sulieman, S. M. Alawad, M. A. Osman, and E. A. Abdelmageed, "Physicochemical Characteristics of Local Varieties of Tamarind (Tamarindus indica L), Sudan," *Int. J. Plant Res.*, vol. 5, no. 1, pp. 13–18, 2015, doi: 10.5923/j.plant.20150501.03.

[3] A. A. Ayat *et al.*, "The effect of blending of extracts of Sudanese Adansonia digitata and Tamarindus indica on their antioxidant, anti-inflammatory and antimicrobial activities," *J. Pharmacogn. Phyther.*, vol. 11, no. 2, pp. 28–34, 2019, doi: 10.5897/jpp2019.0537.

[4] A. P. Menezes, S. C. Trevisan, S. M. Barbalho, and E. L. Guiguer, "Tamarindus indica L. A plant with multiple medicinal purposes," *Phytochemistry*, vol. 5, no. 3, pp. 50–54, 2016.

[5] Zohrameena S, Nesar A, and Saba P, "Medicinal uses & pharmacological activity of Tamarindus indica," *World J. Pharm. Sci.*, pp. 121–133, 2017, [Online]. Available: http://www.wjpsonline.org/

[6] A. A. Adeola, O. O. Adeola, and O. O. Dosumu, "Comparative analyses of phytochemicals and antimicrobial properties of extracts of wild Tamarindus indica pulps," *African J. Microbiol. Res.*, vol. 4, no. 24, pp. 2769–2779, 2010.

[7] P. A. Bressiani, G. R. F. De Lima, E. Düsman, and L. T. D. Tonin, "Cytotoxic and antioxidant activities of Tamarindus indica pulp extract from Brazil," *J. Food Meas. Charact.*, vol. 15, no. 3, pp. 2743–2749, 2021, doi: 10.1007/s11694-021-00855-4.

[8] M. A. A. Ahmed, A. A. A. Mohammed, and K. E. A. Ishag, "Identification and determination of organic acids in some selected medicinal plants grown in Sudan.," *J. Med. Plants Stud.*, vol. 10, no. 1, pp. 12–16, 2022, doi: 10.22271/plants.2022.v10.i1a.1355.

[9] N. Isnaini, S. Songkro, N. Kaewnopparat, D. Maneenuan, and N. Tanmanee, "Formulation and Investigation of Antioxidant Potential of O/W Lotions Containing Tamarindus indica L. Fruit Pulp Extract," *MATTER Int. J. Sci. Technol.*, vol. 5, no. 2, pp. 100–112, 2019, doi: 10.20319/mijst.2019.52.100112.

[10] H. Zhu, Y. Wang, Y. Liu, Y. Xia, and T. Tang, "Analysis of flavonoids in Portulaca oleracea L. by UV-vis spectrophotometry with comparative study on different extraction technologies," *Food Anal. Methods*, vol. 3, no. 2, pp. 90–97, 2010, doi: 10.1007/s12161-009-9091-2.

[11] A. Lamien-Meda *et al.*, "Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso," *Molecules*, vol. 13, no. 3, pp. 581–594, 2008, doi: 10.3390/molecules13030581.

[12] I. Rajhi, M. Abderrabba, N. Besbes, M. T. Ben Dhia, and S. Ayadi, "Solvent extraction effects on phytochemical contents profiles and antioxidant activity of Capparis spinosa leaves," *Phytochem BioSub J. Vol.*, vol. 15(3), no. 2170–1768, 2021, doi: DOI:10.163.pcbsj/2021.15.-3-263.

[13] M. Mohammed, S. Abde, M. A. Abddrahman, and E. Ahmed, "Phytochemical screening , total phenolics content and antioxidants activity of Cassia Singueana," *J. Med. Plants Stud.*, vol. 3, no. 5, pp. 160–165, 2015.

[14] P. R, P. kumar P, and K. S, "GC-MS Analysis of Phytochemicals and Simultaneous Determination of Flavonoids in Amaranthus caudatus (Sirukeerai) by RP-HPLC," *J. Anal. Bioanal. Tech.*, vol. 03, no. 05, pp. 3–6, 2012, doi: 10.4172/2155-9872.1000147.

[15] S. Farooq *et al.*, "Phytochemical investigation and antioxidant activities of tamarind (Tamarindus indica L.)," *Not. Bot. Horti Agrobot. Cluj-Napoca*, vol. 50, no. 4, pp. 1–14, 2022, doi: 10.15835/nbha50412892.

[16] W. Aboshora *et al.*, "Effect of extraction method and solvent power on polyphenol and flavonoid levels in Hyphaene Thebaica L Mart (Arecaceae) (Doum) fruit, and its antioxidant and antibacterial activities," *Trop. J. Pharm. Res.*, vol. 13, no. 12, pp. 2057–2063, 2014, doi: 10.4314/tjpr.v13i12.16.

[17] S. Keser, S. Celik, S. Turkoglu, Ö. Yilmaz, and I. Turkoglu, "Hydrogen Peroxide Radical Scavenging and Total Antioxidant Activity of Hawthorn," *Chem. J.*, vol. 02, no. 01, p. 912, 2012.