Research Article

In vitro antioxidant activities and quantitative chemical composition of alcohol-based extracts of *Adansonia digitata* fruit pulp: A comparative study

Daniel Ayandiran Aina, Kehinde Oluwakemi Fagbemi

Department of Microbiology, School of Science Technology, PMB 4005, Babcock University, Ilisan Remo, Ogun State, Nigeria

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Abstract

Objective: This study aimed at comparing the antioxidant potential, quantitative phytoconstituent and functional group of 70% methanol (70/30 methanol-water) and 70% ethanol solvent used for extraction of phytochemicals from *Adansonia digitata (A. digitata)* fruit pulp. **Material and methods:** The antioxidant analysis was performed with three different methods namely, DPPH, FRAP and TAC, gas chromatography-mass spectrometry (GCMS) analysis was carried out to identify the different types of compounds present in both extraction products, and functional group determination was done with the aid of Fourier Transform Infrared Spectrophotometer (FTIR). **Results and conclusion:** The methanol extract shows excellent antioxidant potential more than the ethanol extract in all the three methods applied, the quantitative analysis results exhibited the presence of 23 and 46 compounds for the ethanol and methanol extract respectively. Furthermore, the FTIR spectrum indicated that both extracts contain compounds with different functional groups. However, the methanol extract shows better absorption intensity than the ethanol extract. The pharmacological activities of the recognized compounds were identified, while the activities of some are unknown. Methanol is found as the most suitable solvent for extracting high numbers of different phytochemical compounds and antioxidants from the fruit of *A. digitata* use in pharmacognosy.

Keywords: Antioxidant, phytoconstituent, *Adansonia digitata,* gas chromatography-mass spectrometry

Introduction

Several human health challenges such as atherosclerosis, cancer, arthritis, ischemia, central nervous system injury, gastritis, cardiovascular diseases, and diabetes have been associated with oxidative stress and autoxidation of human lipids and lipoproteins which eventually leads to the development of free radicals in the body system (Yen et al., 2018; Truong et al., 2019). Consequently, man has sought different ways to alleviate the problem using synthetic antioxidants such as tertbutylhydroquinone, butylated hydroxytoluene, and butylated hydroxyanisole. However, most of these compounds have been discouraged due to their negative side effect, therefore researchers are on a quest for natural antioxidant agents to be used as a substitute for synthetic antioxidants (Złotek et al., 2016). The growing desire for the use of natural antioxidants has resulted in the evaluation of different species of plants parts and products including fruits, leaves, roots, and barks (Santos and Gonçalves, 2016). Recently many synthetic drugs have been produced from medicinal plants due to their effective antioxidant potentials (Chebbac et al., 2021).

Fruits are known to have a high nutrient density score; given this, the World Health Organization (WHO, 2002) recommended the daily consumption of a minimum of 400g of fruit per person. One of the benefits often derived from fruits is the presence of natural antioxidants. According to researches on epidemiological studies, the relationship between fruit and vegetable consumption is inversely related to the risk of having oxidative stress diseases (Ghazzawi et al., 2021). Moreover, nutritional supplements are produced from aromatic plants due to the presence of natural antioxidants, which are known to be

Address for Corresponding Author:
Kehinde Oluwakemi Fagbemi
Department of Microbiology, School of Science Technology, PMB 4005, Babcock University, Ilisan Remo, Ogun State, Nigeria
Email: olakemk4u.kf@gmail.com

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anti-inflammatory agents, preventing inflammation in the human body and thus some inflammatory diseases (Stanković et al., 2016; Yen et al., 2018).

A. digitata L. is an aromatic wild plant that belongs to the family of Malvaceae. It is an indigenous multipurpose tree called many names such as monkey bread tree, symbol of the earth, Ethiopian sour gourd, cream of tartar, African baobab, and chemist tree by both the English and the French (Kamatou et al., 2011). The tree is known to have nine (9) different species in which six (6) *Adansonia grandidieri*, *A. madagascariensis*, *A. perrieri*, *A. rubrostipa*, *A. suarezensis*, and *A. za* are endemic to Madagasscar, and two species namely *A. digitata* and *A. kilima* are found in African while the last one *A. gregorii* is natural to northwestern Australia. Several parts of the tree have been reported to serve as food and are also used for medicinal purposes due to the numerous bioactive compounds present in it. Many ailments and diseases such as diarrhoea, fever, malaria, toothache, gingivitis, cough, and microbial diseases have been treated with the product of this tree. The fruit is known to contain 10 times vitamin C compared to oranges (Asogwa et al., 2021). Many biological activities such as immuno-stimulant, analgesic and anti- Pyretic activity, anti-inflammatory, anti - Trypanosomal activity, insect repellent, and pesticide properties have also been documented concerning the tree (Li et al., 2017).

The first and important process in drug discovery is the extraction of bioactive compounds. The extraction process's main goal is to reduce the number of focus compounds to get the maximal number of bioactive compounds from the process. Factors like the extraction method and the solvent used are responsible for the biological activities exhibited by the extract (Ajanal et al., 2012). Today, a wide variety of solvents such as n-Hexane, Chloroform, methanol, ethylacetate, ethanol, butanol, acetone, and water have been used for the extraction of phytochemicals in plants materials and each resulting to different bioactive compounds extracted. Due to the presence of numerous biologically active compounds in plant samples and the different solubility rates in different solvents, the appropriate solvent to be used for extraction depends on the type of plant materials and the specific compounds to be isolated (Mahdi-Pour et al., 2012). However, the chemical composition and the antioxidant activities using different alcohol-based solvent remains vague and the pharmacological efficiency is required to be probed. Based on all available facts, to the best of my knowledge, there is no sufficient comparative information about the antioxidant potential, chemical composition and functional group of methanol and ethanol extract of baobab fruit pulp. As a result, the purpose of this study is to assess the impact of two alcohol-based solvents (70 per cent methanol and 70 per cent ethanol) on chemical composition using FTIR analysis, gas chromatography-mass spectrometry (GC-MS), as well as the antioxidant capacity of *A.digitata* fruit pulp.

Materials and Methods

Plant collection and preparation

The fruit gourd of *Adansonia digitata* was collected from the wild in April 2019 from Yola Adamawa State, Nigeria. The fruit was ethnobotanically validated in the Department of Botany, and the voucher specimen (number LUH: 8772) was deposited in the herbarium of the University of Lagos, Nigeria. The fruits were sun-dried and broken to remove the pulp. The seeds were manually removed. Eighty grams of pulverized baobab fruit pulp was dispersed into 640 ml of 70% methanol and was agitated occasionally for 72 h for proper digestion. The residue was discarded after it was filtered through a Whatman number 1 filter paper (Saher et al., 2019). The filtrate was kept at 4 °C for further analysis. The same method was applied for the ethanol solvent extraction process.

Fourier Transform Infra-Red Spectroscopy

The Fourier transform infrared spectrophotometer (FTIR) is one of the most powerful tools for determining the types of chemical bonds (functional groups) in compounds. This analysis was performed at Redeemer University Remo, Ogun State, Nigeria, using an infrared spectrophotometer (Perkinelmer, spectrum bx) with a scan range from 400 to 4000 cm-1 with a resolution of 4cm-1. Briefly, 1um each of the pure extract of the two different solvents were deposited on fused sodium chloride (NaCl) cell, it was carefully put on a clamped cell and fixed to an infrared beam. The infrared results obtained was compared to the IR frequencies table (Oladunmoye et al., 2018).

Quantitative analysis of fruit extract

The phytoconstituent of the fruit extract were determined on a Shimadzu GC MS QP 2010 ultra with the following operating conditions: The preliminary temperature was set to 60o^{c}/2mins with a high speed and the final injector temperatures were set to 250o^{c}. During the process of analysis, spitless mode injection was used, helium gas was used as the carrier at the flow rate of 2ml/min and the operating pressure was 144.4Kpa. The retention indices (RI) along with their mass spectral fragmentation patterns were used for the chemical identification. The detected compounds were further confirmed using the NIST/EPA/NIH mass spectrum collection of the National Institute of Standards and Technology (NIST) (2014). To discover the known biological actions of the identified compounds, extensive searches were conducted utilizing

"The PubChem Project" (https://pubchem.ncbi.nlm.nih.gov/), Dr. Duke, and several ethnobotanical databases.

Antioxidant activities

Three different antioxidant evaluation techniques will be used in this present study. The techniques include DPPH radical scavenging activity, ferric reducing power assay (FRAP), and Total antioxidant capacity (phosphomolybdenum) method was selected to determine the antioxidant activities of the methanol and ethanol fruit extracts of Baobab pulp. All reactions were performed with three independent replicates, and each sample was tested three times.

DPPH radicals scavenging activity

DPPH (2,2-diphenyl-2-picrylhydrazyl hydrate) is a very simple, inexpensive, and widely used method of evaluating antioxidant activities. The activity of the DPPH radical is accessed by the reduction of its absorbance value at 517nm. During the analysis, scavenging activities of the radicals were virtually observed by colour changing from purple to yellow. Briefly, 1mL solution was taken from a freshly prepared DPPH methanol stock solution and added to 3mL of the extract solution at different concentrations (2, 4, 6, 8, 10µg/ml). The mixture was vigorously shaken and incubated for 30mins. Then UV-visible spectrophotometer was used to measure the absorbance at 517nm. Gallic acid was used as a standard and the assay was done in triplicate (Shekhar and Anju, 2014). The percentage (%) inhibition exhibited by the extract was used to determine the antioxidant activities using the following formula:

$$\text{DPPH scavenged (\%)} = \frac{(A_{Con} - A_{test})}{A_{Con} \times 100}$$

A_{con} - *is the absorbance of the blank*

A_{test} - *is the absorbance of the extracts.*

Ferric Reducing Antioxidant Power assay (FRAP)

The ferric reducing antioxidant power (FRAP) assay was performed according to the method of Benzie and Strain's (1996) with minor modifications. The primary goal of this method is focused on the reduction of Fe^{3+} to Fe^{2+}. Briefly, varying concentrations of extracts (2, 4, 6, 8, 10µg) was dissolved in 1ML of distilled water and were mixed with 2mL of freshly prepared FRAP reagent which consists of 500ml of acetate buffer with molarity of 300 mM and pH 3.6, 50ml of 2, 4, 6- Tri (2-pyridyl)-s-triazin (TPTZ) (10 mM), and 50 ml of FeCl3. 6H20. The reaction resulted to a deep blue complex of Fe^{2}- and optical density was measured spectrophotometrically against the blank at 593nm using Gallic acid as the standard. The gallic acid equivalent per gram of dry weight (mg GAE/g dry weight) was used to calculate the reducing potential percentage.

Total Antioxidant Capacity (TAC)

The potential of the fruit extract to decrease molybdate ion was determined by the quantitative method described by Prieto et al, 1999. This method focuses on the ability of the extract to reduce Mo (VI) to Mo (V) which will eventually lead to the formation of bluish-green phosphate/Mo (V) compounds. Varying concentration (2,4,6,8 and 10ug) of 0.1mL of the test sample was mixed with 1mL of the Molybdate reagent solution. The mixtures were incubated at 95ºC for 90 min, then allow to cool to room temperature. Then the absorbance was spectrophotometrically measured against blank (methanol) at 695nm.

Statistical Analysis

All analyses were performed in triplicate, and these results were then presented in tables and figures as means with standard deviation where applicable. One-way analysis of variance (ANOVA) was used to compare the results, and *p* values less than 0.05 were considered statistically significant.

Results

The results of the antioxidant activity of *A. digitata* fruit extract determined by the DPPH assay are displayed in Figure 1. The methanol extract was shown to have a better scavenging potential than the ethanol extract, at 4ug, both the methanol and the gallic acid used as standard have a scavenging potential (60%). The ethanol extract was consistently low when compared to others. The radical scavenging activity show an increasing trend with the concentration in this study

The result of the TAC assay displayed in Figure 2 below shows an increasing trend in the concentration of both

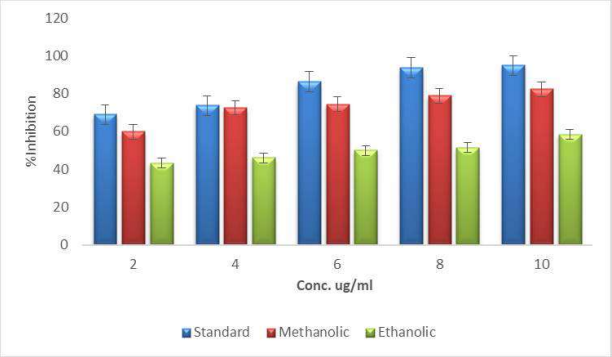

Figure 1. DPPH (% Inhibition) (Mean + Standard deviation) of the *A. digitata*. Note: There is a significant difference in the average scavenging potentials of the *A. digitata*

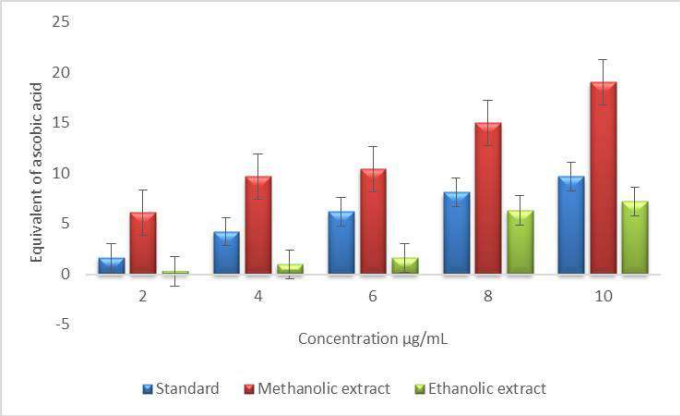

Figure 2. The reduction of molybdenum (VI) to molybdenum (V) equivalent to ascorbic acid (Mean + Standard deviation) by the methanol and ethanol extract of *A. digitata* fruit pulp. There is a significant difference ($\rho < 0.05$) in the average scavenging ability of *A.digitata*. The methanol extract exhibited the highest reducing power.

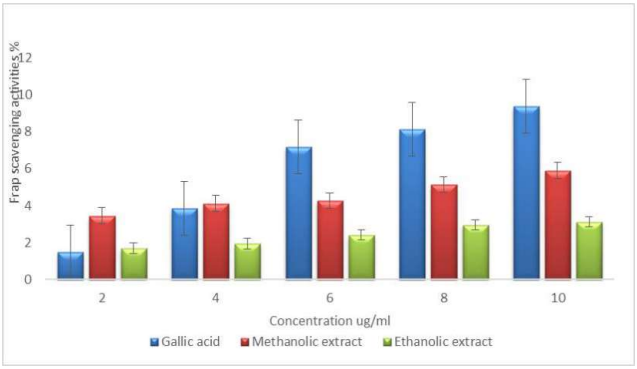

Figure 3. FRAP activity (%) (Mean + Standard deviation) of the ethanol and methanol extract of *A. digitata*.fruit pulp. Note: Based on the same concentration of fruit extract, there is a significant difference ($p< 0.05$) in the average scavenging ability of *A. digitata* fruit pulp.

tested extracts. The methanol extract exhibited antioxidant significant activity than the standard used in the method. At the concentration of 10ug, the methanol extract was two times more effective than the standard.

The result of the FRAP method shows similar activity with the other methods. The methanol extract shows better scavenging activity but is not as high as the standard. The ethanol was consistently low in this method (Figure 3)

Figures 4 and 5 show peaks of different compounds identified by GCMS analysis both chromatograms have 5-Hydroxymethylfurfural as the highest constituent, which is displayed with the highest peak in both chromatograms. The comparison of the chromatograms shows that the same compounds identified from both extracts have different retention times, and their percentage area confirmed the effect of the different solvents used.

The chemical composition of both extracts obtained from *A. digitata* fruit samples, based on the GCMS result as tabulated in Tables 1 and 2 looks a bit similar due to the presence of some compounds. Four compounds are found in both methanol and ethanol, this includes n-Hexadecanoic acid. Sucrose, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, and maltol. However, the compounds are in different concentrations in both extracts. Tables 1 and 2 displayed the pharmacological activities of several of the discovered chemical compounds found in each solvent used. While 26 compounds were identified in ethanol extract, the methanol eluted 46, almost twice as many

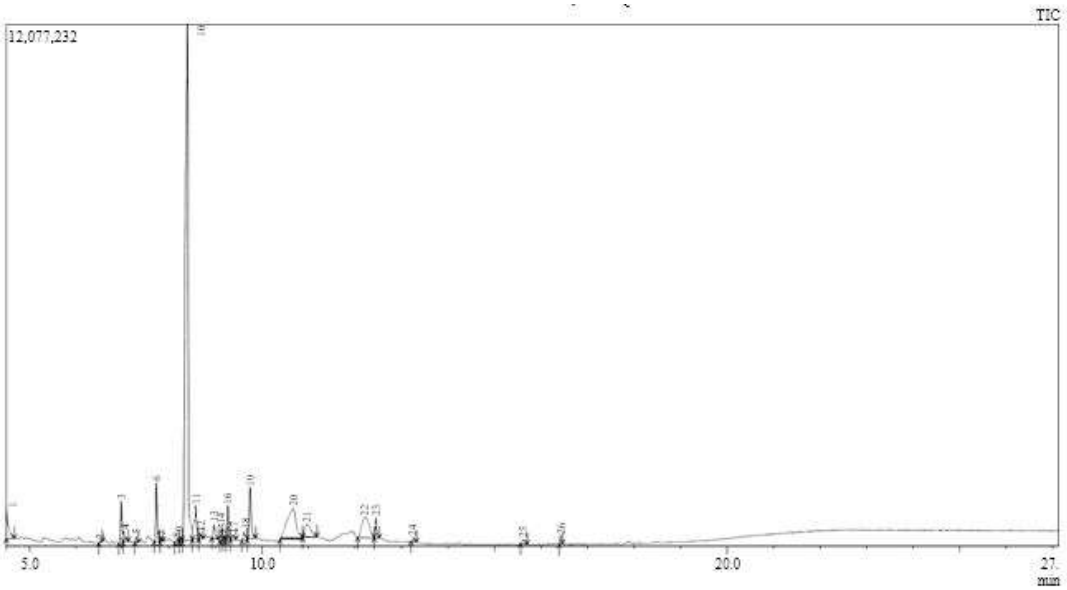

Figure 4. Chromatogram of *A. digitata* ethanolic fruit extract

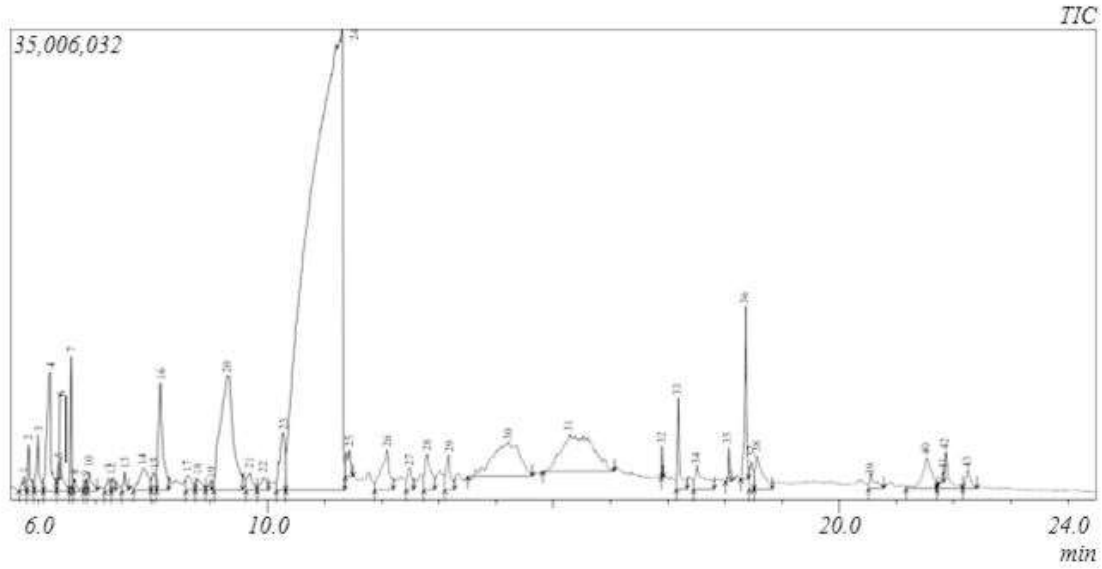

Figure 5. Chromatogram of *A. digitata* methanolic fruit extract

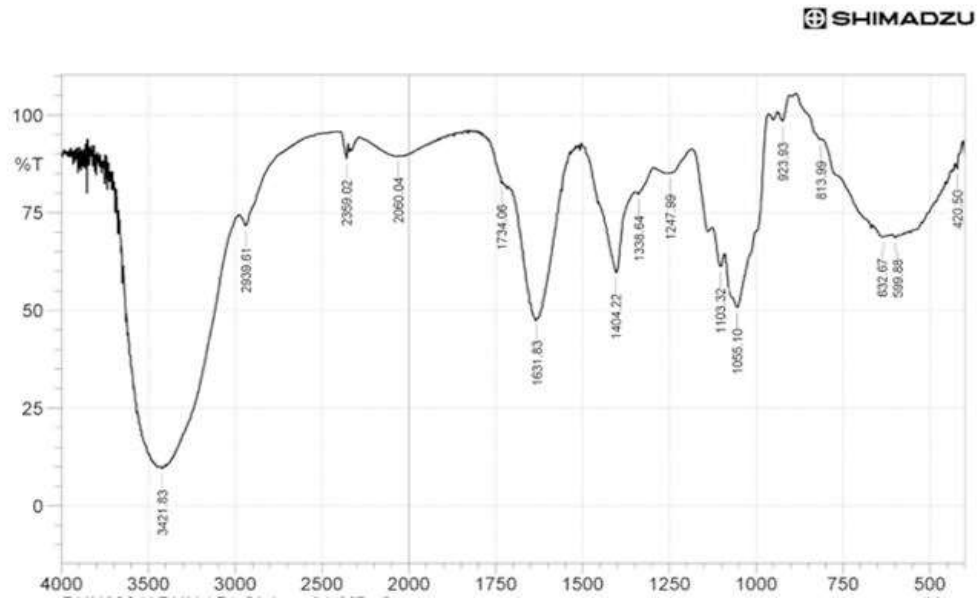

Figure 6. FTIR spectrum of methanol extract of *A. digitata* fruit extract

compounds in ethanol extract. Many of the identified compounds have pharmacological activities, however, the activities of some are unknown. Of all these compounds, 5-Hydroxymethylfurfural has the highest percentage in both the methanolic and ethanolic extract with 61.41% and 54.94% respectively.

The FT-IR spectrum was used for the identification of the functional groups of the potent ingredients present in both extracts are represented in Figures 6 and 7 below. Each spectrum looks similar however, the ethanol spectrum shows more peaks at the fingerprint region than the methanol extract. The results of FT-IR analysis established the presence of N-H, O-H, C=C, C-H, C-O and CH3 functional groups (Table 3)

Discussion

Over the years, there has been an increase in awareness by the scientific community on the use of natural antioxidants and their therapeutic potentials. Plants are known as the main source of natural antioxidants, their antioxidant activities are because they possess reducing power, donate hydrogen ion, quenches singlet oxygen, and chelate metal (Mahdi-Pour et al., 2012; Vujanović et al., 2019). Also, the findings by the World Health Organization (WHO) established that medicinal herbs are used by 80% of the world's population as the main health care. Plants have a very key role in the treatment of malignancy. Antioxidants are very vital substances that have the potential to defend

Table 1. Identified phytocompounds in the ethanol extract of *A.digitata* fruit pulp analyzed by GC-MS

Peak	RT	Area (%)	Name	MW	Formula	Biological Activities	Reference
1	4.505	2.32	But-3-enyl ethyl carbonate	(144.2)	$C_7H_{12}O_3$	Unknown	
2	6.52	0.09	1,3-Dioxol-2-one,4,5-dimethyl-	(114.1)	$C_5H_6O_3$	Potential Inhibitors Against *Vibrio cholera*	El-Naggar et al., 2020
3	6.976	2.69	1,3,5-Triazine-2,4,6-triamine	132.2	$C_3H_{12}N_6$	Antimicrobail agent	Sharma et al., 2017
4	7.035	0.75	Furyl hydroxymethyl ketone	212.3	$C_{14}H_{16}N_2$	Antimcrobial	Popiołek, (2017).
5	7.293	0.25	Propiohydrazide, 2,2-dimethyl-N2-(4,4-dimeth	144.1	$C_6H_8O_4$	Antioxidant, ameliorative, anti-inflammatory	Kumar et al., 2010 Čechovská et al., 2011
6	7.731	3.54	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	318.4	$C_{18}H_{22}O_5$	induced apoptosis	Fehlberg et al 2003,
7	7.845	0.15	Oxirane, 2,2'-[oxybis(methylene)]bis-	170.3	$C_{10}H_{18}O_2$	Antimicrobial and antioxidant	Dr. Duke
8	8.155	0.13	2-Oxepanone, 7-methyl-	212.3	$C_{14}H_{16}N_2$	Antimcrobial	Popiołek,(2017).
9	8.248	0.28	Valeric acid, 4-tridecyl ester	284.5	$C_{18}H_{36}O_2$	Inflammatory	Anitha & Miruthula, (2014).
10	8.4	54.94	5-Hydroxymethylfurfural	126.1	$C_6H_6O_3$	Antioxidant and genotoxic	Coppock, (2021).
11	8.575	3.53	1H-Azonine, octahydro-1-nitroso-	156.2	$C_8H_{16}N_2O$	Carcinogenic	Fan et al., 2018
12	8.68	0.46	Isosorbide Dinitrate	236.1	$C_6H_8N_2O_8$	Visodialator	Holt & Pang, (2019).
13	8.971	1.1	Heptanoic acid, 6-oxo-	144.2	$C_7H_{12}O_3$	Antiviral	Sriram et al., 2018
14	9.131	0.58	Decanoic acid, 3-methyl-	186.3	$C_{11}H_{22}O_2$	Methyl-Guanidine-Inhibitor	Dr. Dukes
15	9.19	0.16	Maltol	126.1	$C_6H_6O_3$	Antimicrobial	Saud, et al., 2019.
16	9.271	1.71	1-Amino-4-methylpiperazine	115.2	$C_5H_{13}N_3$	antiproliferative **activity**	Pogorzelska et al., 2017
17	9.385	0.45	Butanedioic acid, 2-hydroxy-2-methyl-, (S)-	148.1	$C_5H_8O_5$	Antifugal and prevent skin wrinkle	Wu et al., 2020
18	9.616	0.75	2-Thiopheneacetic acid, tridecyl ester	324.5	$C_{19}H_{32}O_2S$	Unknown	
19	9.746	4.3	2-Furanmethanol, tetrahydro-, acetate	144.2	$C_7H_{12}O_3$	Flavouring agent	Pimenta et al., 2018
20	10.674	10.48	Sucrose	342.3	$C_{12}H_{22}O_{11}$	Antibacterial	Zhao et al., 2015
21	10.973	3.16	.beta.-D-Glucopyranose, 1,6-anhydro-	162.1	$C_6H_{10}O_5$	Anti-bacteria and antioxidant activity	Jahan et al., 2020
22	12.227	5.88	d-Glycero-d-galacto-heptose	210.2	$C_7H_{14}O_7$	Antibacteria activity	Monika & Kaur, 2016
23	12.449	1.27	Diethyl Phthalate	222.2	$C_{12}H_{14}O_4$	Antimicrobial activity	Premjanu, & Jaynthy, 2014.
24	13.254	0.29	D-Fructose, 3-O-methyl-	194	$C_7H_{14}O_6$	Antitumor activities	Dr. duke
25	15.617	0.41	7,8,12-Tri-O-acetyl ingol	492.6	$C_{26}H_{36}O_9$	Antibacteria	Al-Rubaye et al., 2017
26	16.415	0.33	n-Hexadecanoic acid	256.4	$C_{16}H_{32}O_2$	Anti-inflammatory activities	Mensah-Agyei et al, 2020

Dr. Duke: Dr. Duke's Phytochemical and Ethnobotanical databases; MW: Molecular weight; RT: Retention Time

the body from damage triggered by free radical-induced oxidative stress (Saeed et al., 2012). A wide variety of pathological diseases are due to the presence of free radicals in the body. The mechanism of action of antioxidants is to scavenge the reactive oxygen species produced by free radicals also averting the formation of peroxide (Čulum et al., 2021). In healthy cells, the production of reactive oxygen species is unavoidable, but this happens at a regulated rate. However, there is a high upsurge in the formation of reactive oxygen species during oxidative stress conditions, leading to subsequent damages in some vital components of the cells. These damages are linked to a variety of health issues, neurodegenerative disorders, and ageing events (Stanković et al., 2016). To sustain a balance redox system and defend the body against excessive reactive oxygen species (ROS) production, humans have searched for several phytotherapeutic substances, which work to prevent harmful impacts of oxidative stress (Muthoni et al., 2020). For quick screening of biomolecules, a wide variety of techniques have been applied in vitro in the evaluation of antioxidant activity, since in vivo antioxidant activities depend on its in vitro efficacy. For instance, compounds with high antioxidant potential in vitro will probably exhibit high activity in vivo. Three different antioxidant techniques were performed to assess the antioxidant potential of Baobab fruit pulp in this study. For the DPPH analysis, both baobab fruit extracts

Table 2. Showing list of identified phytocompounds in methanolic extract of *A.digitata* fruit by GC-MS analysis

Peak	RT	Area (%)	Name	MW	Formula	Biological Activities	References
1	4.505	2.32	But-3-enyl ethyl carbonate	144.2	$C_7H_{12}O_3$	Unknown	
2	6.52	0.09	1,3-Dioxol-2-one,4,5-dimethyl-	114.1	$C_5H_6O_3$	Potential Inhibitors Against Vibrio cholera	El-Naggar et al., 2020
3	6.976	2.69	1,3,5-Triazine-2,4,6-triamine	132.2	$C_3H_{12}N_6$	Antimicrobial	Sharma et al., 2017
4	7.035	0.75	Furyl hydroxymethyl ketone	212.3	$C_{14}H_{16}N_2$	Antimicrobial	Popiołek,2017
5	7.293	0.25	Propiohydrazide, 2,2-dimethyl-N2-(4,4-dimeth	144.1	$C_6H_8O_4$	Antioxidant, ameliorative , anti inflammatory	Kumar et al., 2010; Čechovská et al., 2011
6	7.731	3.54	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-	318.4	$C_{18}H_{22}O_5$	Induced apoptosis	Fehlberg et al., 2003,
7	7.845	0.15	Oxirane, 2,2'-[oxybis(methylene)]bis-	170.3	$C_{10}H_{18}O_2$	Antimicrobial and antioxidant	Dr. Duke
8	8.155	0.13	2-Oxepanone, 7-methyl-	212.3	$C_{14}H_{16}N_2$	Antimicrobial	Popiołek, 2017.
9	6.825	0.09	3,4-Dihydro-6-methyl-2H-pyran-2-one	112	$C_6H_8O_2$	Anticonvulsant and Antimicrobial Activities	Aytemir et al., 2004.
10	6.883	0.32	9-Oxa-bicyclo[3.3.1]nonane-1,4-diol	158	$C_8H_{14}O_3$	Anti-inflammatory activities	Kadhim, (2016).
11	7.225	0.18	2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	112	$C_6H_8O_2$	Antimicrobial, Anti-inflammatory, Anticancer	Pavani & Naika, 2021.
12	7.294	0.14	4-Cyclopentene-1,3-diol, trans-	100	$C_5H_8O_2$	Reverse transcript inhibitor	Boyle et al., 2012
13	7.505	0.21	4-Nonene	126	C_9H_{18}	Not reported	
14	7.835	0.86	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	128	$C_6H_8O_3$	Increases T helper cell and Hepatocarcinogenic	Dr. Duke
15	8.003	0.29	5-Methyl-2-pyrazinylmethanol	124	$C_6H_8N_2O$	Methyl-Guanidine-Inhibitor	Dr. Duke
16	8.13	2.04	Bicyclo[2.2.1]heptane-2-carboxylic acid iso	140	$C_8H_{12}O_2$	HIV inhibitors	Danilenko et al., 2000
17	8.611	0.39	4-Octen-3-one, 6-ethyl-7-hydroxy-	170	$C_{10}H_{18}O_2$	Testosterone-Hydroxylase-Inducer	Dr. Duke
18	8.783	0.29	Pentanoic acid, heptyl ester	200	$C_{12}H_{24}O_2$	Inhibit Production of Uric Acid	Dr. Duke
19	8.992	0.15	trans-2,3-Epoxynonane	142	$C_9H_{18}O$	Reverse-Transcriptase-Inhibitor	Dr. Duke
20	9.304	6.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox	144	$C_6H_8O_4$	Anti-proliferative and pro-apoptotic effects.	Roy et al., 2018
21	9.697	0.44	Allyl heptanoate	170	$C_{10}H_{18}O_2$	insecticidal and repellent properties	Giner et al., 2013
22	9.93	0.37	Cyclohexanol, 3,3,5-trimethyl-	142	$C_9H_{18}O$	Skin irritation	Belsito et al., 2008
23	10.28	1.31	3-Methyl-2-furoic acid	126	$C_6H_6O_3$	Antcancer	Zawadzki, et al., 2020
24	11.29	61.41	5-Hydroxymethylfurfural	126	$C_6H_6O_3$	Anti-inflammatory activity and inhibits DNA polymerase γ and is nephrotoxic	Coppock, 2021. Sharma et al., 2014
25	11.43	0.44	Cyclohexanone, 2-isopropyl-2,5-dimethyl-	168	$C_{11}H_{20}O$	Antifungal activities	Floris et al., 2021
26	12.09	1.33	Maltose	342	$C_{12}H_{22}O_{11}$	Immunostimulatory action in HIV infection	Patel et al., 2020
27	12.48	0.41	4-Hydroxy-3-methylpent-2-enoic acid, meth	144	$C_7H_{12}O_3$	Anticancer	Dr. Duke
28	12.79	0.89	3-Hexenoic acid, 5-hydroxy-2-methyl-	144	$C_7H_{12}O_3$	Hexokinase-Stimulator	Dr. Duke
29	13.16	0.64	(Z),(Z)-2,5-Dimethyl-2,4-hexadienedioic ac	144	$C_7H_{12}O_3$	unknown	
30	14.21	3.84	Stevioside	804.88	$C_{38}H_{60}O_{18}$	Anti-oxidants, and antimicrobial properties	Favaro, Rocha-Selmi, & dos Santos (2015).
31	15.27	4.79	.alpha.-D-Glucopyranoside, O-.alpha.-D-gl	342	$C_{12}H_{22}O_{11}$	Antibacterial activity	Kamal et al., 2015.
32	16.88	0.12	Cyclopentanetridecanoic acid, methyl ester	297	$C_{19}H_{36}O_2$	Antimicrobial and antioxidant activities	Suseem, & Saral, (2013).
33	17.17	0.84	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-Alpha reductase inhibitor	Abubaka, & Majinda, (2016).
34	17.5	0.88	9-Oxabicyclo[6.1.0]non-6-en-2-one	138	$C_8H_{10}O_2$	Unknown	

Table 2. Continue.....

35	18.05	0.2	9,12,15-Octadecatrienoic acid, methyl ester	292	$C_{19}H_{32}O_2$	Antiinflammatory, nematicide, insectifuge, antiacne, Hypocholesterolemic, Cancer preventive, HepatoprotectiveAnti inflammatory activities	Godwin et al., 2015
36	18.35	1.42	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$	Anticancer, antibacterial, antioxidant, antipyretic, cardioprotective	Godwin et al., 2015
37	18.44	0.38	17-Octadecynoic acid	280	$C_{18}H_{32}O_2$	Anticancer, antibacterial, antioxidant, antipyretic, cardioprotective	Godwin et al., 2015
38	18.55	1	cis-Z-.alpha.-Bisabolene epoxide	220	$C_{15}H_{24}O$	Alpha-glucosidase inhibitors21	Mishra & Patnaik, 2020.
39	20.54	0.35	Hexadecanoic acid, 2-hydroxy-1-(hydroxym	331	$C_{19}H_{38}O_4$	Aphrodisiac activity	Ganesh, & Mohankumar, 2017
40	21.518	1.19	4,22-Stigmastadiene-3-one	411	$C_{29}H_{46}O$	Hemolytic, pesticide, flavour, antioxidant.	Tyagi, & Agarwal, 2017.
41	21.79	0.09	9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hyd	355	$C_{21}H_{38}O_4$	Anticancer	Mohammed 2019
42	21.85	0.81	Butyl 9,12,15-octadecatrienoate	334	$C_{22}H_{38}O_2$	Anti-inflammatory	Ebin, 2021.
43	22.238	0.37	beta.-Sitosterol	415	$C_{29}H_{50}O$	Antioxidant, anticancer, anti-diabetic, antimicrobial and immunomodulatory activities.	Evangelina et al., 2021; Babu & Jayarama (2020

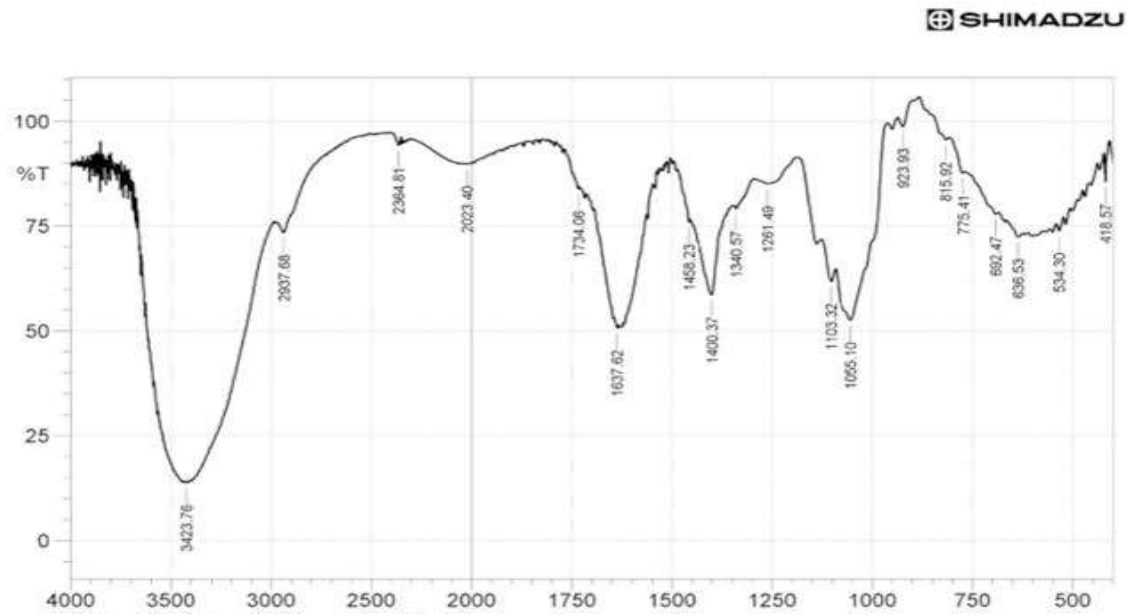

Figure 7. FTIR spectrum of ethanol fruit extract of *A. digitata*

show to possess antioxidant agents however, the methanolic extract exhibited more radical scavenging activities than the ethanolic extract (Fig 1). This report is consistent with earlier reports on the activities of these two solvents on phytochemical extraction on fruits (Truong et al., 2019; Venkatachalam et al., 2020; Zaman et al., 2020). Conversely

Table 3. FTIR spectral peak values and functional groups of both ethanol and methanol extract of *A. digitata* fruit

Spectrum No.	Ethanol Extract Wavenumber cm^{-1}	Methanol Extract Wavenumber cm^{-1}	Functional group	Predicted compounds
1	3423.761 S	3421.83 S	O-H Stretching	Hydroxyl compounds
2	2937.69 W	2939.61W	Asymmetric stretching of–$CH(CH_2)$ vibration	Lipid, Amines
3	2364.81 W	2359.02 W	C=-CH Bending	alkyne
4	2023.40 W	2060.04 W	Symmetric stretching vibration of C=-N	Free amino acid
5	1734.06 W	1734.06 W	Asymmetric vibration of C=O in esters	Fatty acid
6	1637.62 M	1631.83 M	C=N stretching vibration	Aromatic compounds
7	1458.23 W	1404.22 M	C-H stretching	Aromatic compounds
8	1400.37 M	1338.64W	Stretching vibration of C-H	Aromatic compounds
9	1340.57 W	1247.99 W	C-H	Alkanes
10	1261.49 W	1103.32W	Stretching of C-O	Alcohol, Carboxylic acid, Ester
11	1103.32 M	1055.10 M	C=O	Alcohols, Ether, Carboxylic acid
12	1055.10 M	923.93 W	Stretching of C=O	Aliphatic compounds
13	923.93 W	813.99 W	Stretching of C-O	Carboxyl
14	815.92 W	632.67 M	Stretching of C-H	Aromatic compounds
15	775.41W	599.88 M	C-H	Aromatic compounds
16	692.47 W	420.50W	C-H	Aromatic compounds
17	636.53 W	----------	C-H	Aromatic compounds
18	534.30 W	----------	Asymmetric bending of C-C-N	Nitriles
19	418.57 W	----------	Asymmetric bending of C-C	Cycloalkanes

Note: S means strong; M means medium and W means weak absorption intensity

to the findings of Iloki-Assanga et al., 2015. where ethanol displayed more antioxidant activities in *Bucida buceras* L. and *Phoradendron californicum*. The antioxidant capacity of both the ethanolic and methanolic solvent was determined spectrophotometrically through phosphomolybdenum method, The formation of the complex green colour was observed due to the reduction of molybdenum (VI) to molybdenum (V). Both extracts exhibited an increase in TAC activity as the concentration increased however, the methanol shows twice potency more than the ethanol and better activity than even the gallic acid used as standard (Figure 2).

In Figure 3, the reducing ability of the ferric reducing antioxidant activity of the methanolic extract compared against ethanolic extract and gallic acid showed a significant difference ($\rho < 0.05$) and it increases with an increase in concentrations. The methanolic displayed the highest FRAP activity than that of ethanolic extract and even Gallic acid. The FRAP result obtain in this study corroborates the findings of Ndiaye et al. (2021), where the methanolic extract of baobab fruit also exhibited high antioxidant activity by the Frap method. However, this is contrary to the report of Do et al. (2014) where ethanol extract of Limnophila aromatic spice gave better antioxidant activities than methanol. Overall, the methanolic extract of *A. digitata* displayed the best antioxidant properties. The antioxidant result obtained in this study was supported by the GC-MS analysis of the extract, as the most abundant compound identified in both the methanol and ethanol extracts is 5-Hydroxymethylfurfural, which has the highest peak as shown in the chromatogram (Figures 4 and 5). This compound has been earlier reported in *A.digitata* fruit by Tembo et al., 2017 and Ismail et al., 2021. Also, many pharmacological activities such as antioxidant, genotoxic, nephrotoxic, and anti-inflammatory potential have been attributed to it (Sharma et al., 2014; Coppock, 2021). However, it must be noted that, that the biological activities of

plant extracts cannot be limited to the presence of a single compound. Many activities displayed by plant extract are by the synergistic effect of the different compounds present (Cai et al., 2016; Pezzani et al., 2019).

Out of the 26 compounds observed by the GC-MS profiling of the ethanolic extract illustrated in Table 1, the most prominent compounds are 5-Hydroxymethylfurfural (54.94%), Sucrose (10.48%) and d-Glycero-d-galacto-heptose (5.88%) 2-Furanmethanol, tetrahydro-, acetate (4.3%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-(3.54%) and 1H-Azonine, octahydro-1-nitroso- (3.53%), which all displayed a wide range of biological activities (Table1). While for the methanol extract, (Table 2) 43 compounds were observed and the most prominent compounds are 5-Hydroxymethylfurfural (61.14%), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox (6.14%), Alpha. -D-Glucopyranoside, O- alpha-D-gl (4.79%), Stevioside (3.84%) and Bicyclo [2.2.1] heptane-2-carboxylic acid iso (2.04%). Pharmacological activities of many of these compounds are listed out in table 1 above, although the activities of some of the compounds such as 6-Oxa-bicyclo [3.1.0] hexan-3-one, 4-Nonene. (Z), (Z)-2,5-Dimethyl-2,4-hexadienedioic acid, 2-Thiopheneacetic acid, tridecyl ester But-3-enyl ethyl carbonate, and 9-Oxabicyclo [6.1.0] non-6-en-2 one is not yet reported. Some compounds such as n-Hexadecanoic acid. Sucrose, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6, maltol are all present in both extracts but in a very small quantity. Hexadecanoic acid also knowns as palmitic acid have been well recognized by many authors to have antioxidant, anticancer, and anti-inflammatory activities (Mensah-Agyei et al., 2020; Abiodun et al., 2020). 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 is another compound with many biological activities including ameliorative effects (Olaniyan et al., 2018). Antimicrobial, anti-inflammatory (Kumar et al., 2010). Other minor compounds such as 6-Oxa-bicyclo [3.1.0] hexan-3-one, 8-Oxabicyclo [5.1.0] octane, 4-Cyclopentene-1,3-diol, trans, 5-Methyl-2-pyrazinylmethanol, trans-2,3-Epoxynonane, Allyl heptanoate, Z),(Z)-2,5-Dimethyl-2,4-hexadienedioic acid, 9,12,15-Octadecatrienoic acid, methyl ester, cis-Z-.alpha.-Bisabolene epoxide , beta.-Sitosterol etc were discovered in the methanol extract of baobab fruit pulp while the ethanol extract does not contain any of such compounds. Previous reports (Rehman et al., 2019; Pepple et al., 2020) also discovered the presence of 9,12,15-Octadecatrienoic acid, methyl ester in the methanolic extract of their plants. A similar result was obtained by Sermakkani and Thangapandian, (2012) when working on the extraction of *Cassia italica* leaf extract using methanol as the solvent. Based on the pharmacological activities of these compounds reported in literature, some of them possessed antioxidant potential and therefore the higher antioxidant activities reported from the fruit pulp are likely linked to the presence of these compounds. It is noteworthy to know that the type of solvent used for extraction determines the type of secondary metabolite eluted. In this study, methanol exhibited excellent activities because it has a higher dielectric constant than ethanol, and this enables the extraction of more polar compounds. Moreover, it also has a lower boiling point (64.7 °C) while ethanol boils at 78.4 °C. Many bioactive compounds would have been denatured during the process of evaporation with ethanol (Borges et al., 2020).

Lastly, the presence of different bioactive compounds was further confirmed with the aid of Fourier transform infrared spectrometry (FT-IR). This equipment shows the presence of the functional groups that are available in both extracts. Extracts from both solvents contain similar functional groups such as amines, phenols, alcohols, carboxylic acids, alkanes, aliphatic compounds, carbonyl compounds, esters, and aromatic compounds as seen in the GCMS results. The data obtained was used to identify the functional group of the active components and analyzed based on the interpretation of Coats et al., 2000. The FTIR spectrum was illustrated in Figures 6 and 7 and assignments of bands to functional groups are tabulated in Table 3. The FTIR spectra results confirmed the presence of the following groups: O-H, NH2, -CH, -CH3, -COOH, -C=O, -C-O, -C-C, -CH, and -C-C in both but the absorption intensity of the bands in the ethanol solvent was weaker than that of the methanol. Similar peak characteristics were observed with the spectrum of these two solvents; however, the ethanol spectrum showed a little difference in the fingerprint region, the slight variation in their spectrum could be attributed to the ability of each solvent to extract different pharmacologically active components present in the fruit extract, which collaborates our findings from the GCMS results and it is consistent with the findings of Subashini et al. (2015) who found that these functional groups indicate the existence of secondary metabolites and other bioactive components in plants and its products.

Conclusion

This study demonstrated the impact of the extraction solvent on the phytoconstituent and antioxidant potentials of *A. digitata* fruit pulp. Moreover, the findings of this study give scientific proof of the folkloric usages of this wild fruit. Effective extraction procedures could result in the development of therapeutic agents with the plant-based origin and many other nutraceutical products which can have a positive influence on human health. Additional

biological activity-guided techniques are essential to separate the probable active compounds from their bulk material.

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