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Antioxidant Potential And GC-MS Profiling of an Underutilized Wild Edible Fruit *Maesobotrya barteri* (Baill) Hutch

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Abstract

Maesobotrya barteri fruit is commonly consumed in southern Nigeria, owing to its medicinal and nutritional benefits. This study investigated the phytochemical composition, antioxidant activity, and GC-MS profile of methanol and aqueous extracts of *M. barteri* fruit. The antioxidant properties of both extracts were assessed using DPPH radical scavenging activity, ferric reducing antioxidant power (FRAP), and metal-chelating activity assays. Phytochemical screening revealed saponins, tannins, flavonoids, and cardiac glycosides in both extracts, whereas alkaloids and anthraquinones were absent. The total phenolic and flavonoid content ranged from 12.82 ±1.03 mg GAE/g to 15.75 ± 0.87 mg GAE/g and 49.10 ± 1.33 mg QE/g to 156. 3 ± 3.14 mg QE/g, respectively. Antioxidant analysis showed IC_{50} values for DPPH (74.88 ± 2.86, 113.30 ± 2.98, and 17.18 ± 0.61 µg/mL), FRAP (27.11 ± 1.55, 23.28 ± 0.85, and 38.15 ± 1.33 µg/mL), and metal chelating activity (71.12 ± 2.01, 135.96 ± 3.22, and 101.98 ± 2.08 µg/mL) for the methanol extract, aqueous extract, and ascorbic acid, respectively. GC-MS analysis identified 17 and 14 compounds in methanol and aqueous extracts, respectively. The major compounds in the methanol extract were glycerol, 6-oxa-bicyclo [3.1.0] hexan-3-one, erythritol, D-allose, and esters of hexadecenoic, octadecanoic, and octadecatrienoic acid. The aqueous extract was rich in glycerol, erythritol, 3,4tetrahydrofurandiol, cyclohexanone, and 5-hydroxymethyl furfural. The presence of these bioactive compounds highlights the potential applications of *M. barteri* fruits in phytomedicine and nutraceuticals.

Keywords: *Maesobotrya barteri*, fruit extract, total phenolic and flavonoid, antioxidant activity, GC-MS analysis

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Introduction

Phytomedicines are gaining popularity for their therapeutic, nutritional, and nutraceutical applications. The therapeutic potential of these plants is primarily assessed by identifying their bioactive constituents, which often include a variety of compounds such as flavonoids, alkaloids, terpenoids, tannins, glycosides, and anthraquinones (Siddiqui *et al.*, 2023; Rodríguez-Negrete *et al.*, 2024; Gupta *et al.*, 2025). Flavonoids comprise a broad range of compounds, from simple flavones to more complex anthocyanidins (Wojcik *et al.*, 2010; Panche, 2016). Alkaloids are among the largest groups of secondary metabolite heterocyclic compounds, recognized for their diverse biological activities. These compounds range from basic indole pyrrolidine to intricate vinblastine alkaloids, which are used in the treatment of lymphoma and breast cancer (Matsuura and Fett-Neto, 2015). Saponins, tannins, terpenoids, and glycosides exhibit diverse and unique systems and have found biological activities, including antioxidative, antiplasmodial, antimicrobial, anti-inflammatory, anticancer, antidiabetic, and hypolipidemic (Alhassan *et al.*, 2017; Saini *et al.*, 2022; Fakudze *et al.*, 2023). Phytomedicines have demonstrated efficacy in the treatment of various health conditions including chronic infections (Enin *et al.*, 2005; Enin *et al.*, 2024a), mental health disorders (Wubetu *et al.*, 2018; Pferschy-Wenzig *et al.*, 2022), cardiovascular diseases (Adegbola *et al.*, 2017; Rouhi-Boroujeni *et al.*, 2017), cancer (Khan *et al.*, 2019; Ohiagu, 2021), and diabetes (Enin *et al.*, 2023; Enin *et al.*, 2024b). The biological activities of these plants are intricately tied to their structural diversity, highlighting the importance of studying their structures as crucial tools for predicting their bioactivity (Tillotson *et al.*, 1996; Roy *et al.*, 2018). In addition to their direct therapeutic use, these bioactive compounds have various functions as nutraceuticals, UV filters, detoxifiers, osmoregulatory agents, enzyme inhibitors, and dietary supplements (Biharee *et al.*, 2020; Dwivedi *et al.*, 2020). One of the key features of these compounds is their ability to neutralize free radicals and alleviate oxidative stress, which is linked to various diseases, such as diabetes, cancer, cardiovascular issues, inflammation, thyroid disorders, and neurodegenerative diseases (Huy *et al.*, 2008; Pisoschi and Pop, 2015). These classes of compounds are considered to be antioxidants.

Antioxidants help prevent the formation of reactive oxygen species, capture harmful radicals, repair damaged nucleic acids, remove oxidized proteins, and restore oxidized lipids using enzymes such as hydrolases and phospholipases (Serafini *et al.*, 2006; Sen and Chakraborty, 2011; Sardesai, 1995). Free radicals, such as reactive oxygen, nitrogen, and chlorine, are major causes of health problems globally. These highly reactive entities can harm nucleic acids, proteins, enzymes, and other vital biomolecules, ultimately disrupting their structure and function (Kowalczyk, 2013; Phaniendra *et al.*, 2015; Ifeanyi, 2018). Intense research on oxidative pressure is ongoing, owing to the alarming rate of stress-related deaths. The current interest is focused on fruits, vegetables, and spices as affordable, ecofriendly, and less toxic sources of antioxidant agents against oxidative stress and cohorts. *Maesobotrya barteri* (Figure 1) is a shrub plant belonging to the Euphorbiaceae family, which is the third largest genus of flowering plants, housing approximately 2000 species that are native to several African regions, including the rainforest areas of Sierra Leone, Southern Nigeria, and Western Cameroon (Etukudo, 2003; Ubulom *et al.*, 2017; Mikailu and Ifeachukwu, 2019). Commonly referred to as "squirrel cherry" in English and "nyanyated" by the Ibibio people of Akwa Ibom State, Nigeria, it bears juicy white berries, though some reports mention a black-purple variety. Ethnopharmacologically, plants have been used to treat diabetes, malaria, dysentery, arthritis, mumps, and rheumatism (Ubulom *et al.* 2017). Its twigs are used as chewing sticks, roots are infused into gins for arthritis treatment, and stems are used for fencing and supporting yam tendrils (Etukudo, 2003; Ubulom *et al.*, 2017). Stem has been reported to have antimicrobial properties (Ogwuche and Edjere, 2016). Phytochemical screening and nutritive and proximate composition of stems and leaves have recently been reported (Ajuru and Wilson, 2024). Despite Nigeria's rich diversity of medicinal and nutritional plants, many of these species remain underutilized due to a lack of information on their nutritional value and medicinal properties, primarily because of insufficient validation of their bioactivity. Thus, this study aimed to investigate the bioactive composition and antioxidant properties of methanol and aqueous fruit extracts of *Maesobotrya barteri* to explore its potential medicinal benefits.


Figure 1: *Maesobotrya barteri* fruits

Materials and Methods

Plant collection and identification

Fresh Maesobotrya barteri fruits were harvested from a forest in Ediene Attai Village in the Oruk Anam Local Government Area of Akwa Ibom State, Nigeria, in March 2024. Plant identification and authentication were performed in the Department of Botany and Ecological Study, Faculty of Biological Sciences, University of Uyo, Nigeria. Fresh fruits were washed with flowing water, sliced, air-dried at ambient temperature for two weeks and reduced into fine powder using a laboratory mill.

Plant Extraction

The method described by Ouandaogo *et al*. (2023) was used to extract plant samples. Ninety grams (90 g) of finely powered fruit was placed in conical flasks and extracted with 70% methanol. The flasks were then placed on a flat-plate mechanical shaker (Platform ZD881) and macerated for 14 hours at 25^0C. The resulting solution was filtered, and the filtrate was concentrated to dryness *in vacuo* to obtain the methanol extract. Another ninety grams (90 g) (90 g) were macerated with water at 65 °C for 3 h to obtain an aqueous extract. Both the methanol and aqueous extracts were weighed, and the percentage yield was calculated.

Preliminary phytochemical analysis

Phytochemical tests for flavonoids, alkaloids, saponins, tannins, cardiac glycosides, and anthraquinones were performed according to standard methods (Ouandaogo *et al*. 2023).

Quantitative Phytochemical Screening

Total Phenolic Content

Total phenolic content was determined spectrophotometrically following a standard procedure (Kim *et al*., 2003). Briefly, the sample (0.5 mL; 1 mg/mL in methanol) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 7% Na_2CO_3. The reaction mixture was vortexed for 15 s and incubated at 40 °C for 30 min in the dark to allow for color development. Absorbance was measured using a UV-Vis spectrophotometer (Techmel and Techmel, USA) at 765 nm. A calibration curve was prepared using gallic acid solutions (10–100 µg/mL), which was determined by extrapolating the sample absorbance values on the standard curve, and the results were expressed as milligrams of gallic acid equivalent per gram dry weight.

Total Flavonoids Content

Total flavonoid composition was determined using the protocol described by Subhashini et al. (2010). The extract solution (1 mg/mL) was diluted with 200 µL distilled water, followed by the addition of 150 µL 5% sodium nitrite ($NaNO_2$) solution. This mixture was incubated for 5 min and then added to 150 µL 10% $AlCl_3.6H_2O$. After 6 min, 2 mL of 1M NaOH was added. Absorbance was measured using a UV-Vis spectrophotometer at 510 nm and the total flavonoid content was expressed as mg of quercetin (QE) equivalent per gram dry weight.

In vitro antioxidant analysis

2.5.1. The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity Assay

DPPH activity was assessed using standard methodology (Shekhar and Anju, 2014). DPPH (1 mL, 0.1 mM) was mixed with 3 mL of a solution

containing the extract and ascorbic acid, and the mixture was stirred for one minute. The mixture was incubated in the dark for 30 min before measuring absorbance at 517 nm using a UV/Vis spectrophotometer (Techmel and Techmel, USA). The percentage of DPPH radical-scavenging activity was calculated using the following equation:

$$\text{DPPH percentage scavenging effect} = \frac{[(A0 - As)]}{[A0]} \times 100$$

Where A0 is the absorbance of the control reaction, and as is the absorbance of the standard.

Ferric Reducing Antioxidant Power (FRAP) Assay

Ferric reducing power was determined according to the method described by Ali *et al*. (2020). Various concentrations (µg\ml) of the extract were added to 1 mL of 200 mM sodium phosphate buffer (pH 6.6) and 1 mL of potassium ferricyanide (0.69 mL) [K_3[Fe $(CN)_6$]. The mixture was then incubated at 50°C for 20 min. Trichloroacetic acid (1 mL, 10%) was prepared and dissolved in 50 mL distilled water. The mixture was then centrifuged at 650 rpm for 10 minutes. The upper layer (4 mL) was mixed with 4 mL of deionized water and 0.8 mL of 0.1% (v/v) anhydrous ferric chloride ($FeCl_3$), and the absorbance was measured using a UV-vis spectrophotometer at 700 nm. This procedure was repeated using various concentrations of ascorbic acid. A higher absorbance indicates a higher reducing power.

$$\text{Mean Abs} = [Abs\ 1 + \text{Abs } 2 + \text{Abs } 3]/3$$

Where Abs = absorbance of the sample

Metal Chelating Activity

The metal-chelating activity of the extracts was determined following a previously described method (Köksal et al., 2009), with minor modifications. A methanol solution of the extract (0.5 mL) and ascorbic acid (0.5 mL) at various concentrations (20–100 µg/mL) was mixed with methanol (3 mL), iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 2 mM, 0.1 mL), and ferrozine (5 mM, 0.2 mL). The mixture was incubated in the dark for 10 min. A blank control was also prepared without the extract. The metal-chelating activity was determined by measuring the absorbance at 562 nm using a UV/Vis spectrophotometer (Tecmel and Techmel, USA).
Metal chelating activity (%) = (1− absorbance of sample/absorbance of control) × 100

GC-MS Analysis.

A GCMS-QP2010SE (SHIMADZU model, Japan) with a column length of 30 m thickness, 0.25 m; and diameter 0.25 mm was employed to analyze the samples. Helium was used as the carrier gas at 1 mL/min, and a sample injection volume of 1µL was at a split ratio (10:1). The oven temperature was taken from 60 °C with an increase of 5 °C/min to 180 °C and subsequently, a ramp of 20 ˚C/min to 250 ˚C. The ion source temperature was adjusted to 230 °C and the ionization voltage was set to 70 eV. The GC-MS data were interpreted using the National Institute of Standards and Technology (NIST) (Kadhim et al., 2016).

Statistical analysis

All experiments were performed in triplicates. Microsoft Excel was used for all statistical analysis. Results are expressed as the mean ± SD.

Results

Extraction

The aqueous extract afforded the highest yield (26.5%), which was approximately twice that of the methanol extract (13.5%).

Phytochemical analysis

Preliminary phytochemical screening of the extracts indicated the presence of saponins, tannins, flavonoids, and cardiac glycosides in the methanol and aqueous fruit extracts, whereas alkaloids and anthraquinones were not detected in any of the extracts (Table 1).

Total Phenolic and Flavonoid Content

The total phenolic content in the extracts ranged from 12.82 ±1.03 mg GAE/g to 15.75 ± 0.87 mg GAE/g, with the aqueous extract exhibiting slightly higher phenolic content (15.75 ± 0.87 mg GAE/g) compared to the methanol extract. For flavonoids, the range was between 49.10 ± 1.33 mg QE/g to 156. 3 ± 3.14 mg QE/g. The methanol extract had a significantly higher flavonoid content (156.3 ± 3.14

mg QE/g), approximately three times more than the aqueous extract (49.10 ± 1.33 mg QE/g) (Table 2).
Antioxidant activity

The 2,2-Diphenyl-1-picrylhydrazyl radical scavenging

Both extracts scavenged DPPH radicals and exhibited reducing potential in a concentration

dependent manner (Figure 2). The methanol extracts scavenged DPPH radicals with a higher inhibition percentage than that of the aqueous extract. At 100 µg/mL, the methanol extract scavenged 57% of DPPH radicals, whereas the aqueous extract scavenged 48% of DPPH radicals. The standard drug (ascorbic acid) exhibited 84% inhibitory activity. At 40 µg/mL, the aqueous extract scavenged 25% of DPPH radicals, which was comparable to the 43% scavenging activity of the methanol extract. The IC_{50} values (Table 2) indicated the following trend: Ascorbic acid (17.18 $\pm$ 0.61 µg/mL) > methanol extract (74.88 $\pm$ 2.86 µg/mL) > aqueous extract (113.30 $\pm$ 2.98 µg/mL).

The Ferric Reducing Antioxidant Power

Ferric reducing power was assessed by measuring the absorbance of each extract and plotting the mean absorbance values against the extract concentrations (µg/mL). Absorbance readings at concentrations between 20-100 µg/mL revealed a dose-dependent reduction in activity (Figure 3). At 40 µg/mL, the absorbance values were as follows: aqueous extract (0.776); methanol extract (0.771); and ascorbic acid (0.645). At the highest concentration (100 µg/mL), the absorbance values increased to 0.827 for the aqueous extract, 0.841 for the methanol extract, and 0.824 for ascorbic acid (Figure 3). The IC_{50} values were: methanol extract (27.11 $\pm$ 1.55 µg/mL), aqueous extract (23.28 $\pm$ 0.85 µg/mL), and ascorbic acid (38.15$\pm$ 1.33 µg/mL), indicating that the aqueous extract displayed the strongest reducing power (Table 2).

The Metal Chelating Activity

Both the methanol and aqueous extracts demonstrated dose-dependent chelating effects on Fe (II), with the methanol extract consistently showing stronger chelation ability than the aqueous extract at all concentrations (Figure 4). At 40 µg/mL, the metal chelation capacities were as follows: methanol extract (49.1%); aqueous extract (26.7%); and ascorbic acid (45.5%). At the maximum concentration (100 µg/mL), the chelation values were as follows: methanol extract (53.0%); aqueous extract (38.7%); and ascorbic acid (52.1%). In terms of the concentration required to chelate 50% of the available Fe (II) (IC_{50}), the methanol extract was the most effective (IC_{50} = 71.12 $\pm$ 2.01 µg/mL). The aqueous extract had an IC_{50} value of 135.96 $\pm$ 3.22 µg/mL, while ascorbic acid had an IC_{50} of 101.98 $\pm$ 2.08 µg/mL.

Gas chromatography-mass spectrometry (GC-MS)

GC-MS analysis revealed a total of 17 compounds in the methanol extract (Table 3), whereas 14 compounds were detected in the aqueous extract (Table 4). The chromatograms are shown (Figures 5 and 6). For the methanol extract, compounds present were: 6-oxa-bicyclo[3.1.0]hexan-3-one (7.43%), βD-glucopyranose, 1,6-anhydro- (4.44%), cyclooctasiloxane, hexadecamethyl- (3.39%), glycerine (26.39%), 1,6-Andro-β-D-glucofuranose (2.78%), cyclopentylcarboxylic acid (1.97%), erythritol (5.66%), D-allose (3.88%), hexadecanoic acid, methyl ester (3.98%), octanoic acid, 2methylphenyl ester (1.24%), hexadecanoic acid, ethyl ester (3.83%), menthol, 1'-(butyn-3-one-1-yl), (1S, 2S,5R)- (1.56%), 9-octadecenoic acid (Z)-, methyl ester (9.68%), 9,12,15-octadecatrienoic acid, ethyl ester (Z, Z)- (4.23%), methyl stearate (2.12%), (E)-9-octadecenoic acid ethyl ester (7.69%), 9,12,15-octadecatrienoic acid, ethyl ester (Z, Z)- (3.59%), octadecanoic acid, ethyl ester (1.21%), 9octadecenoic acid (Z)-, methyl ester (1.16 %) and 9octadecenoic acid (Z)-, methyl ester (3.28%). The results of the aqueous extract revealed the following compounds: cyclohexanone (2.98%), glycerine (62.27%), 3,4-furandiol, tetrahydro-, trans- (3.21%), 1,2,3-propanetriol, 1-acetate (0.49%), 5hydroxymethyfurfural (2.31%), erythritol (19.41%), orcinol (1.48%), 1,6-anhydro-*β*-d-talopyranose (1.29%), D-allose (2.70%), 1,2,4-cyclopentaetrione, 3-butyl- (0.98%), *δ*-1, *α*-cyclohexane acetic acid (1.26%), cyclohexanone, 2-(1-methyl-2-nitroethyl)- (0.62%), undecane, 6-cyclohexyl- (0.53%), furan, tetrahydro-2,2-dimethyl-5-(1-methy-1-propenyl)- (0.41%).

Table 1: Phytochemical analysis of the fruit extracts of *Maesobotrya barteri.*

Test	Methanol Extract	Aqueous Extract
Flavonoids	+	+
Saponins	+	+
Alkaloids	-	-
Tannins	+	+
Cardiac glycosides	+	+
Anthraquinones	-	-

Key: + = present; - =

Table 2: Total phenolic content, flavonoids and antioxidant activity of the extracts

Assay	Methanol	Aqueous	Ascorbic acid
TPC (mg GAE/g)	12.82 ± 1.03	15.75 ± 0.87	-
TFC (mg QE/g)	156.3 ± 3.14	49.10 ± 1.33	-
DPPH IC_{50} (µg/mL)	74.88 ± 2.86	113.30 ± 2.98	17.18 ± 0.61
FRAP IC_{50} (µg/mL)	27.11 ± 1.55	23.28 ± 0.85	38.15± 1.33
Metal Chelating IC_{50} (µg/mL)	71.12 ± 2.01	135.96 ± 3.22	101.98 ± 2.08

Table 3: GC-MS Analysis of methanol fruit extract of *Maesobotrya barteri.*

Peak	Compound	MF	R_t (min.)	Area (%)	MW	SI
1.	6-oxa-bicyclo [3.1.0] hexan-3-one	$C_5H_6O_2$	9.381	7.43	98	88
2.	β-D-glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	10.150	4.44	162	92
3.	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{14}O_8Si_8$	10.289	3.39	592	82
4.	Glycerine	$C_3H_8O_3$	10.740	26.39	92	96
5.	1,6-andro-β-D-glucofuranose	$C_6H_{10}O_5$	12.849	2.78	162	89
6.	Cyclopentylcarboxylic acid	$C_6H_{10}O2$	13.982	1.97	114	59
7.	Erythritol	$C_4H_{10}O_4$	14.401	5.66	122	95
8.	D-allose	$C_6H_{12}O_6$	17.406	3.88	180	93
9.	Hexadecanoic acid, methyl ester	$C17H_{34}O_2$	18.398	3.98	270	97
10.	1,6-andro-β-D-glucofuranose	$C_6H_{10}O_5$	18.521	1.56	162	89
11.	Octanoic acid, 2-methylphenyl ester	$C_{15}H_{22}O_4$	19.102	1.24	234	70
12.	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	19.500	3.83	284	95
13.	Menthol, 1'-(butyn-3-one-1-yl)-, (1S, 2S,5R)-	$C_{14}H_{22}O_2$	20.227	1.56	222	77
14.	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	21.427	9.64	296	96
15.	9,12,15-octadecatrienoic acid, ethyl ester (Z, Z)-	$C_{19}H_{32}O_2$	21.541	4.23	292	96
16.	Methyl stearate	$C_{19}H_{38}O_2$	21.765	2.12	298	97
17.	(E)-9-octadecenoic acid ethyl ester	$C_{20}H_{38}O_2$	22.470	7.69	38	92
17.	9,12,15-octadecatrienoic acid, ethyl ester (Z, Z)-	$C_{20}H_{34}O_2$	22.597	3.59	306	94
18.	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	22.822	1.21	312	93
19.	9-octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	23.278	1.16	296	91
20	1-(4-ethylpiperazin-1-yl) ethanone	$C_8H_{16}N_2O$	26.080	3.28	156	71

MF: Molecular formular; R_t (min.): Retention time in minutes; MW: Molecular formular (g/mol); SI: standard index

Table 4: GC-MS Analysis of aqueous extract.

Peak	Compound	MF	R_t (min)	Area%	MW	SI
1.	Cyclohexanone	$C_6H_{10}O$	9.547	2.98	98	89
2.	Glycerine	$C_3H_8O_3$	10.661	62.27	92	96
3.	3,4-furandiol, tetrahydro-, trans-	$C_4H_8O_3$	11.064	3.21	104	94
4.	1,2,3-propanetriol, 1-acetate	$C5H_{10}O_4$	12.293	0.49	134	.85
5.	5-hydroxymethyfurfural	$C_6H_6O_3$	13.986	2.31	126	87
6.	Erythritol	$C_4H_{10}O_4$	14.565	19.41	122	95
7.	Orcinol	$C_7H_8O_2$	15.845	1.48	124	88
8.	1,6-anhydro-*β*-d-talopyranose	$C_6H_{10}O_5$	16.201	1.29	162	87
9.	D-allose	$C_6H_{12}O_6$	16.915	0.88	180	85
10.	1,2,4-cyclopentaetrione, 3-butyl-	$C_9H_{12}O_3$	17.081	0.98	168	81
11.	D-allose	$C_6H_{12}O_6$	17.446	1.82	180	94
12.	*δ*-1, *α*-cyclohexane acetic acid	$C_8H_{12}O_2$	19.657	1.26	140	76
13.	Cyclohexanone, 2-(1-methyl-2-nitroethyl)-	$C_9H_{14}O_2$	20.614	0.62	154	80
14.	Undecane, 6-cyclohexyl-	$C_{17}H_{34}$	21.671	0.53	238	76
15.	Furan, tetrahydro-2,2-dimethyl-5-(1-methy-1-propenyl)-	$C_{10}H_{18}O$	22.661	0.41	154	71

MF: Molecular formular; R_t (min.): Retention time in minutes; MW: Molecular weight (g/mol); SI: standard index

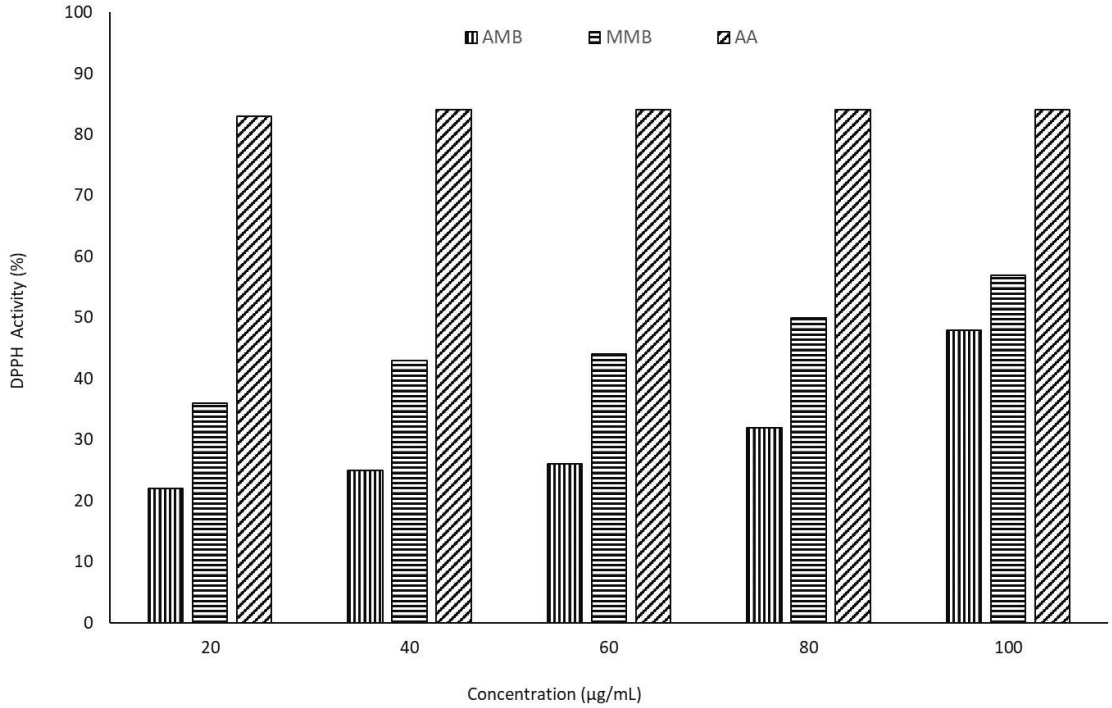

Figure 2: DPPH Radical Scavenging Activity of Methanol and Aqueous Extracts.

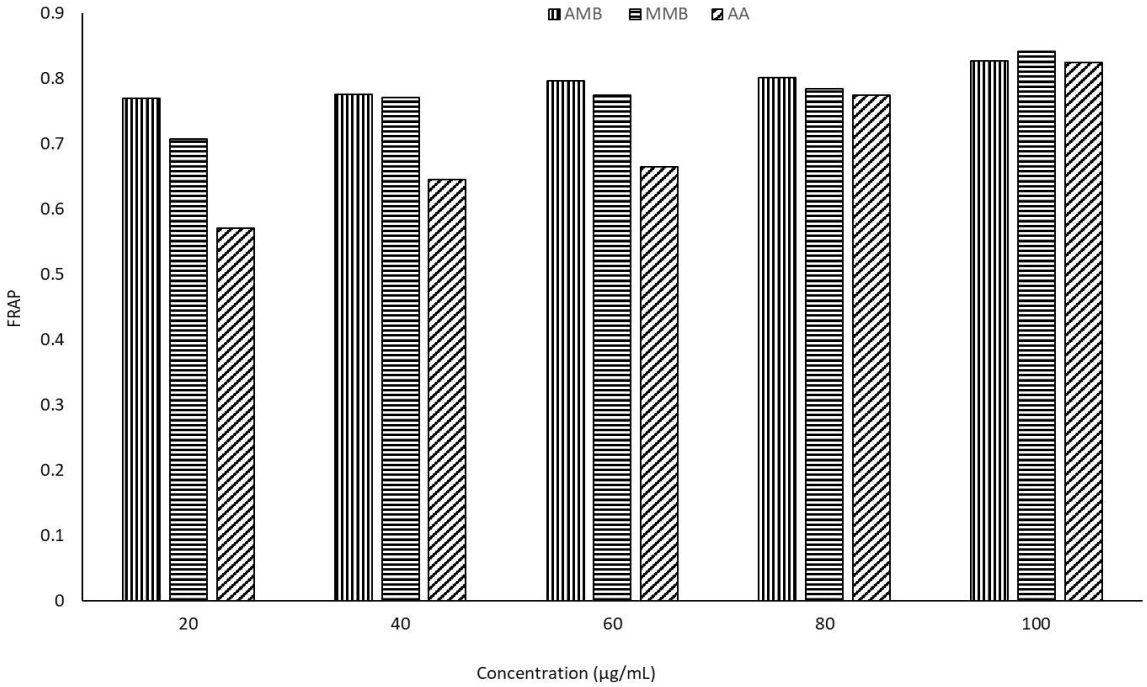

Figure 3: Ferric Reducing Antioxidant Power Assay of Methanol and Aqueous Extracts

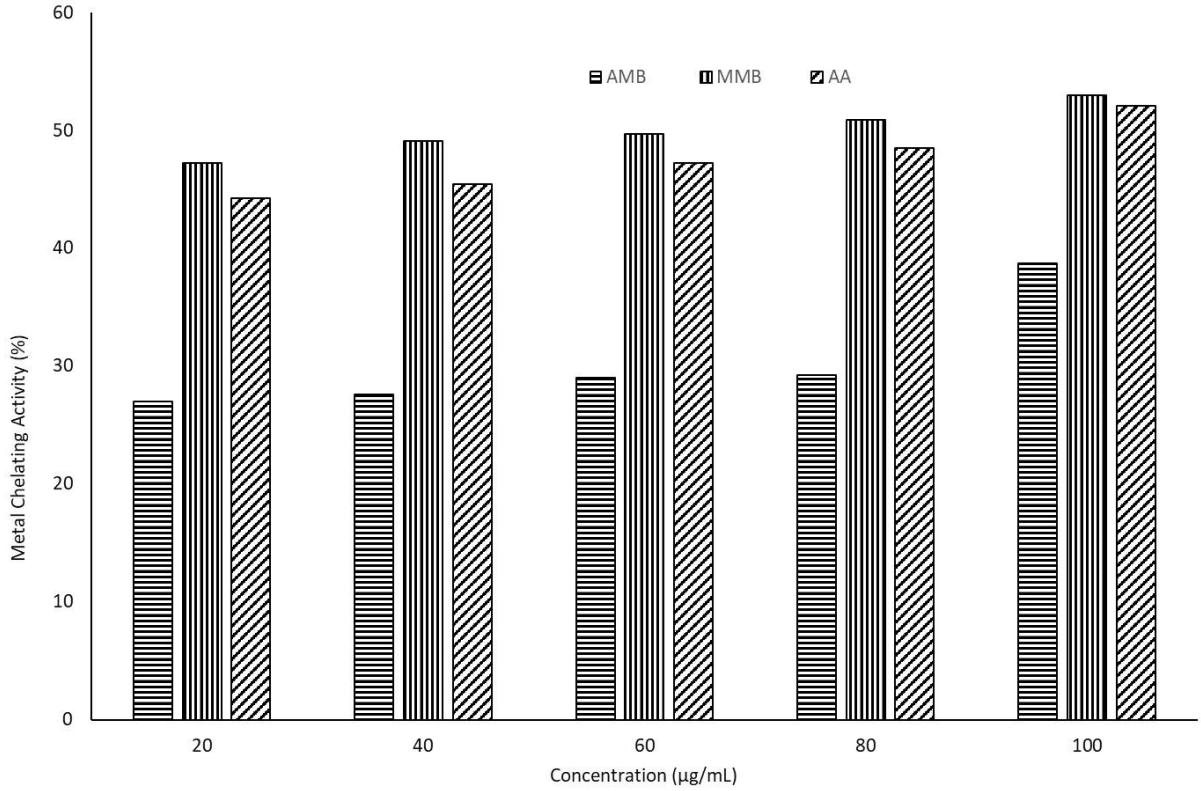

Figure 4: Metal Chelating Activity of Methanol and Aqueous Extracts

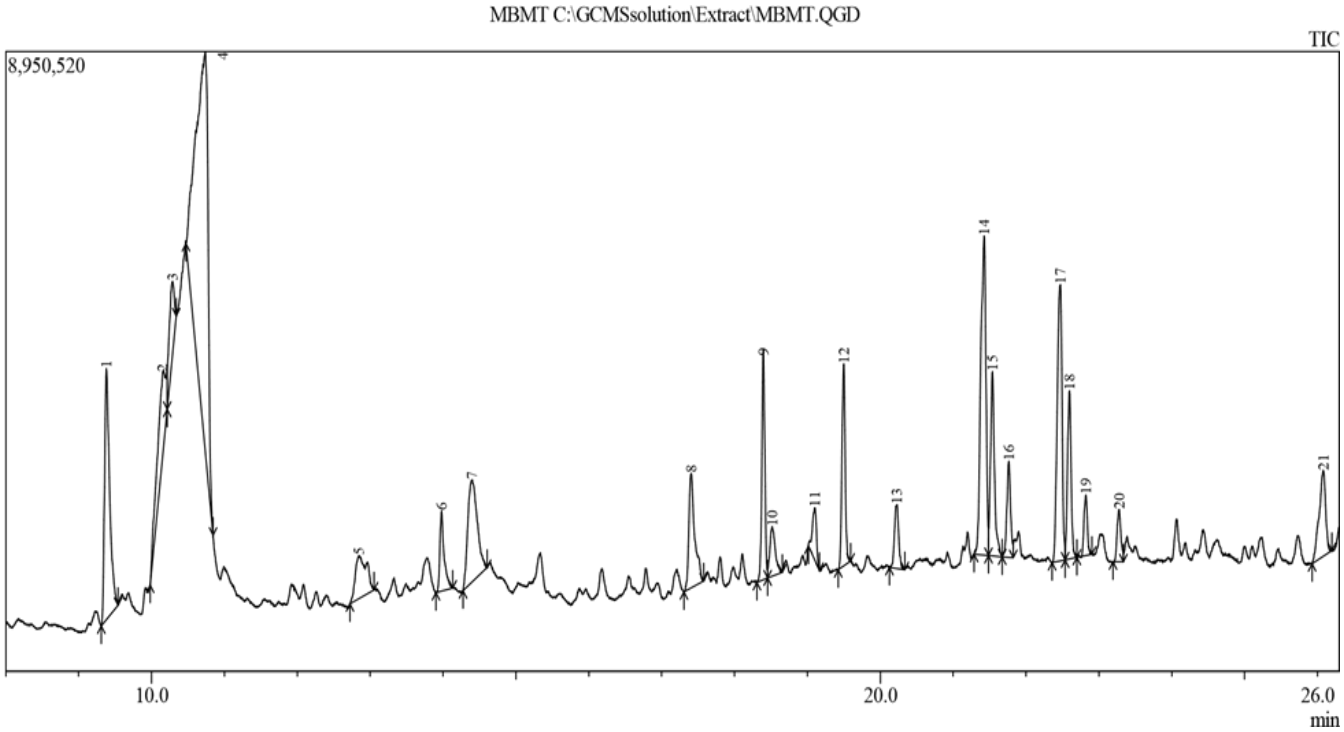

Figure 5: GC-MS Chromatogram of Fruit Methanol Extract of *Maesobotrya barteri.*

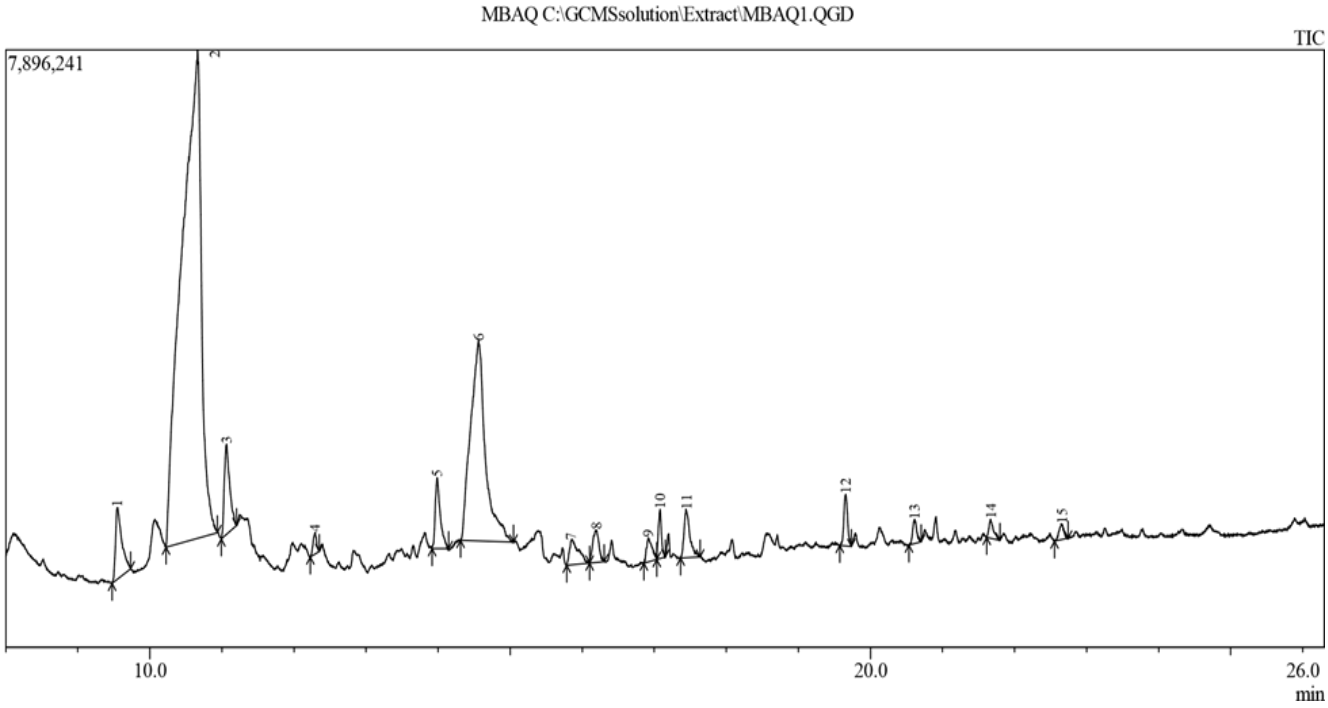

Figure 6: GC-MS Chromatogram of Aqueous Fruit Extract of *Maesobotrya barteri.*

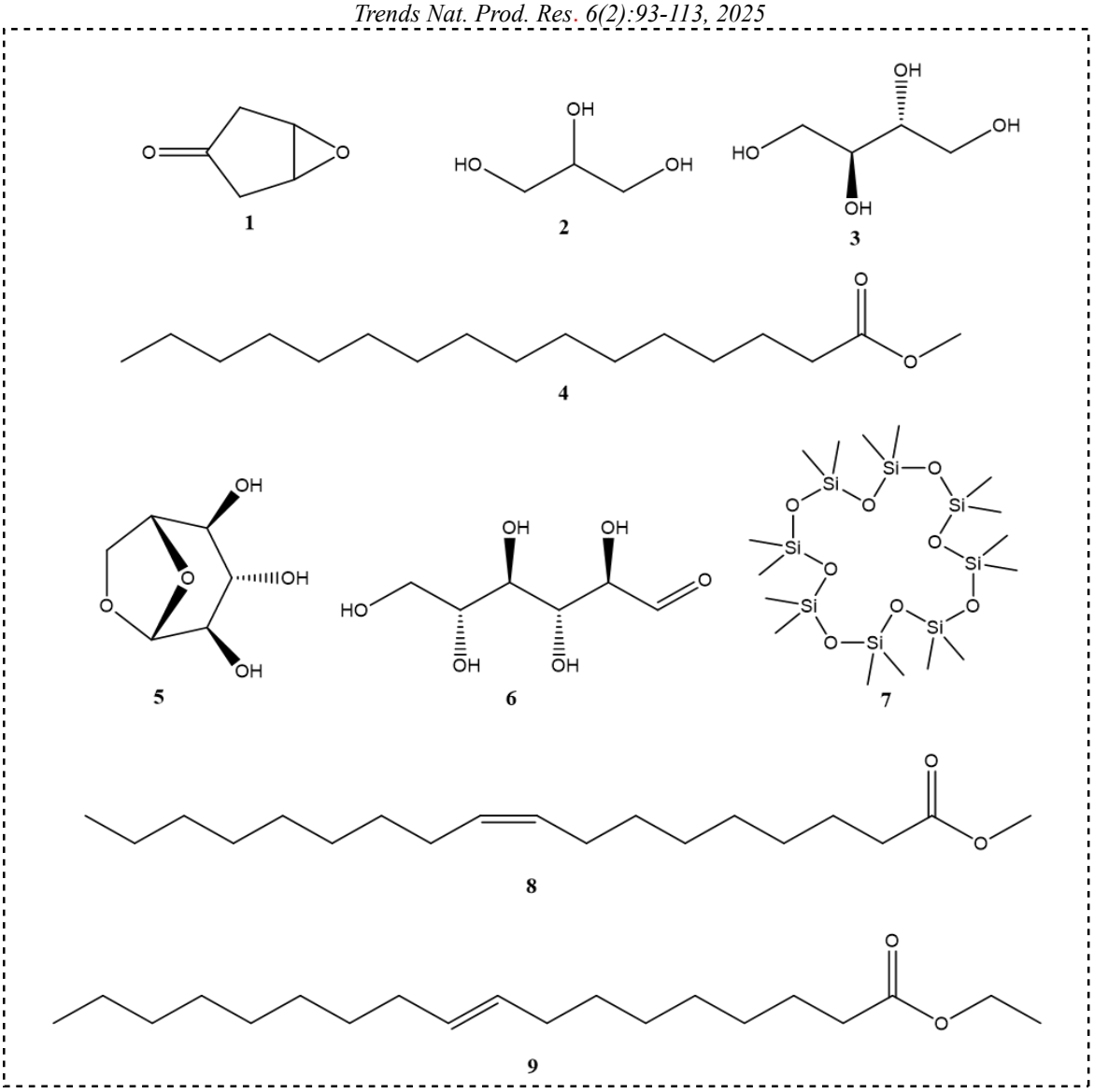

Figure 7: Prominent compounds in the GC-MS analysis of fruit methanolic and aqueous extracts of *Maesobotrya barteri*. *1. 6-oxa-bicyclo [3.1.0] hexan-3-one 2.* Glycerine 3. Erythritol 4. Hexadecanoic acid methyl ester 5. βD-glucopyranose, 1,6-anhydro- 6. D-allose 7. *Cyclooctasiloxane, hexadecamethyl- 8. 9-octadecenoic acid (Z)-methyl ester (9.* (E)-9-octadecenoic acid
ethyl ester.

Discussion

The variation in extraction yields observed in this study could be partly attributed to the polarity of the phytoconstituents. Methanol is capable of extracting both polar and non-polar compounds, unlike water, which mainly extracts highly polar substances, such as enzymes, pigments, and other bioactive compounds, thus achieving the highest extract yield (Bakari *et al.*, 2015). Plant metabolites are known for their diverse bioactivities, including antimalarial, antioxidant, anti-inflammatory, anti-allergic, cardioprotective, and cytotoxic effects (Andreu *et al.*, 2018; Juszczak *et al.*, 2021). Flavonoids have demonstrated antimalarial and anti-inflammatory effects; saponins exhibit cytotoxic and anti-ulcer properties, whereas tannins are known for their antiseptic, antiviral, antifungal, and antimutagenic properties (Mwine and Damme, 2011). The total phenolic and flavonoid contents of the methanol and aqueous extracts suggest that the methanol extract preferentially extracted higher amounts of flavonoids, whereas the aqueous extract had a higher phenolic content. Ahmed *et al.* (2019) reported total phenolic and flavonoid contents of 14.17 mg GAE/g and 1.06 mg QE/g respectively for methanol fruit extract of *Alphitonia philippinensis*, while Orak *et al.* (2019) reported total phenolic

content of 28.36 mg GAE/g and total flavonoid content of 13.95 mg QE/g for methanol fruit pulp extract of *Annona muricata* L. Compared to their findings, our results suggest that *M. barteri* methanolic extract has a lower total phenolic but relatively higher flavonoid content. Previous studies have shown that extraction solvents, plant origin, growth stage, soil nutrients, and climatic conditions contribute to variability in the polyphenolic content of plant extracts (Sukweenadhi *et al.*, 2020). The antioxidant activities of the extracts were evaluated by measuring their DPPH radical scavenging ability, ferric reducing antioxidant potential (FRAP), and metal-chelating ability, which varied in a dose-dependent manner. In comparison to previous studies, lower DPPH scavenging abilities were observed for seasonal fruit extracts from Jordan and aqueous fruit extracts of
Phyllanthus acidus (Andrianto *et al.*, 2019;
Andrianto *et al.*, 2017). The ability of a substrate to form stable iron (II) chelates is linked to its capacity to reduce free ferrous ions, thus mitigating the Fenton reaction, which is a key mechanism of oxidative stress-mediated cellular damage
(Halliwell and Gutteridge, 1984).

Overall, the antioxidant results revealed a significant correlation between the total phenolic and flavonoid content of the fruit extracts. While the methanol extract had a higher flavonoid content, the aqueous extract showed a slightly higher phenolic content, which was consistent with the results from the FRAP, DPPH, and metal chelation assays. This suggested that the antioxidant potential of *M. barteri* is closely related to its polyphenolic constituents. GC-MS analysis of the extracts revealed the presence of ketones, glycosides, saturated and unsaturated fatty acid esters, and alcohol. Notably, two compounds, D-allose and glycerin, were present in both extracts but at different concentrations. The aqueous extract contained a significantly higher concentration of glycerin (62.27%) than the methanol extract (26.33%). In contrast, the methanol extract contained a slightly higher concentration of D-allose (3.88%) than the aqueous extract (2.70%). Fatty acid esters dominated the methanol extract, whereas alcohols and ketones were more abundant in the aqueous extract. The key constituents of the methanol extract were glycerine (26.39%), 9-octadecenoic acid (Z)-methyl ester (9.68%), (E)-9-octadecenoic acid ethyl ester (7.69%), 6-oxa-bicyclo [3.1.0] hexan-3-one (7.43%), erythritol (5.66%), 9,12,15octadecatrienoic acid, ethyl ester (Z,Z) (4.23%), and hexadecanoic acid methyl ester. Glycerin (62.27%) and erythritol (19.41%) were the dominant compounds in the aqueous extract.

Glycerin (glycerol), a triol compound, has diverse industrial and biomedical applications including cosmetics (creams and toothpaste), pharmaceuticals (cough syrups), food additives, biodiesel production, and textiles (Eccles and Mallefet, 2017; Paliagro and Rossi, 2008). It is also used as a plasticizer, cytoprotectant, and solvent in laboratory settings (Mast 1991). Erythritol, a non-caloric polyol (1,2,3,4-butanetetriol), offers endothelial protection and promotes dental health by reducing plaque accumulation (Boesten *et al.* 2015). Additionally, it has antioxidant and antimicrobial properties and is considered safe for patients with diabetes because it has no impact on plasma glucose (Bornet *et al.*, 1996).

Moreover, 9-octadecenoic acid (E)-methyl ester has antioxidant and anticancer properties (Yu *et al.*, 2005); 9,12,15-octadecatrienoic acid ethyl ester (Z, Z)- exhibits anticancer, antibacterial, antipyretic, cardioprotective, and antiarthritic properties (Godwin *et al.*, 2015); and hexadecanoic acid methyl ester exhibits antioxidant, anti-inflammatory, antihyperlipidemic, and antimicrobial effects (Ukwubile *et al.*, 2019). The abundance of bioactive compounds such as glycerin and erythritol in the fruit extracts of *M. barteri* highlights its potential as a candidate for the extraction and development of therapeutic agents. Further studies should focus on isolating these compounds, evaluating their biological activities, and conducting toxicity assessments to determine their pharmacological relevance.

Conclusion

This study highlights the fruit of *Maesobotrya barteri* as a rich source of bioactive compounds including saponins, tannins, flavonoids, and cardiac glycosides. The antioxidant assay confirmed notable activity of both methanol and aqueous extracts, with the methanol extract showing higher DPPH scavenging and metal-chelating effects. GC-MS profiling identified 17 and 14 compounds in the methanol and aqueous extracts, respectively, with glycerol, erythritol, and several esters as the prominent components. These findings suggest that *M. barteri* fruit holds substantial promise as a functional food ingredient or phytomedicinal candidate for combating oxidative stress. Future studies should aim to isolate and characterize individual bioactive compounds and validate their therapeutic potential through further biological and toxicological evaluations.

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Conflict-of-Interest

The authors declare that they have no conflicts of interest.

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