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FULL ARTICLE

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Evaluation of the nutritional composition of *Myrothamnus flabellifolius* (Welw.) herbal tea and its protective effect against oxidative hepatic cell injury

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Abstract

The nutrient composition of *Myrothamnus flabellifolius* leaf tea extract (MLTE) and its protective effect against oxidative hepatic cell injury were evaluated. Gallic acid, caffeic acid, ferulic acid, methyl gallate, and epicatechin were identified in MLTE by high-performance liquid chromatography (HPLC). The tea extract showed an appreciable nutritional content of proximate, sugar, vitamin E, monounsaturated fatty acids, omega 6 and 9 unsaturated fatty acids, as well as considerable amounts of various mineral elements. Nineteen amino acids were found. Moreover, MLTE exhibited potent in vitro antioxidant activities, presumably because of its richness in polyphenols (gallic acid and ferulic acid) and vitamin E. In Chang liver cells, pretreatment with MLTE suppressed oxidative lipid peroxidation (IC_{50} = 113.11 μg/ml) and GSH depletion (IC_{50} = 70.49 μg/ml) without causing cytotoxicity. These data support the local consumption of *M. flabellifolius* herbal tea, which may be used against oxidative stress-induced diseases while providing the body with necessary nutrients.

Practical application

Herbal teas are one of the most consumed beverages in the world today, due to their refreshing taste and additional health benefits. *Myrothamnus flabellifolius* herbal tea is a widely used traditional herbal tea in Southern Africa with potentials for commercialization due to its pleasant flavor. This study, for the first time, reported the nutritional composition of the leaf decoction of *M. flabellifolius* and its protective effect on hepatic oxidative insults. These results can inform the dietary and nutritional use of the tea for optimum benefits, as well as provide preliminary scientific validation of the use of the herbal tea as an antioxidant beverage with good nutritional value.

KEYWORDS

antioxidant, herbal tea, *Myrothamnus flabellifolius*, nutritional composition, polyphenols

Abbreviations: ABTS, 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate); DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; HPLC, high-performance liquid chromatography; MLTE, *Myrothamnus flabellifolius* leaf tea extract; ORAC, oxygen radical absorbance capacity; ROS, reactive oxygen species; TAC, ABTS radical antioxidant capacity.

1 | INTRODUCTION

Oxidative stress is pivotal in the initiation, progression, and associated complications of several diseases because of physiological imbalance between pro-oxidants and antioxidants (Pizzino et al., 2017). There are several physiological processes such as oxygen metabolism, lipid peroxidation, and external factors like xenobiotics, pollutants, and UV radiation that lead to the excessive production of pro-oxidants and reactive oxygen species (ROS) (Pizzino et al., 2017). On the other hand, the body is equipped with antioxidant mechanisms to annul the oxidative effects caused by these pro-oxidants on proteins, lipids, and nucleic acids (Chukwuma & Islam, 2017). When the ROS production is excessive, oxidative damage to biological molecules occurs that leads to several pathological conditions or complications such as diabetes, hypertension, cardiovascular diseases, cancer, rheumatism, neurodegenerative diseases, and the aging process (Pizzino et al., 2017). Hence, antioxidant molecules like polyphenols and certain vitamins are known for their role in the management and prevention of oxidative stress-induced diseases, such as cancer, atherosclerosis, Alzheimer's disease, rheumatoid arthritis, and diabetic nephropathy (Landete, 2013; Pizzino et al., 2017).

Most medicinal plants, in particular, the herbal infusions and teas, are rich sources of polyphenols and vitamins, which have been reported in previous studies to possess appreciable antioxidant properties and ameliorative effects against oxidative stress (Landete, 2013). For example, green tea characteristically contains catechins, epicatechins, epigallocatechin-3-gallate, epigallocatechin, and epicatechin-3-gallate, which have strongly influenced their consumption due to their health benefits (Chacko, Thambi, Kuttan, & Nishigaki, 2010).

In South Africa, herbal teas from *Aspalathus linearis* (Rooibos tea), *Cyclopia intermedia* (Honeybush tea), and *Hibiscus sabdariffa* (Roselle or red tea) are the most exported teas with a growing global market. Several studies have confirmed their health benefits, which have been attributed to the presence of xanthone, mangiferin, flavonones, aspalathin, dihydrochalcones, nothofagin, and other polyphenols. However, preparation methods, processing, and fermentation have been stated to affect their nutritional composition and antioxidative capacity (Von Gadow, Joubert, & Hansmann, 1997). Bhebhe, Chipurura, and Muchuweti (2015) showed that the antioxidant properties and total phenolic content recorded in herbal teas of *Myrothamnus flabellifolius* (Welw.), *Fadogia sycamore* L., and *Fadogia ancylantha* (Schweif) cultivated in Zimbabwe are higher than South Africa rooibos tea, which has become popular globally. Therefore, there is a need for further investigation on their nutritional property, especially *M. flabellifolius*, a widely used traditional herbal tea in Southern Africa (SANBI, 2013).

M. flabellifolius (Welw.) (Family: Myrothamnaceae) is a shrub commonly referred to as "resurrection bush" or locally as "Moritela Tshwene" in the North West Province of South Africa and Botswana, because the dried leaves quickly rehydrate and appear fresh and green when it rains (Viljoen et al., 2002). It is indigenous to Southern Africa with a wide distribution in several provinces in South Africa, such as Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and the North West, where it grows on sunny rocky north-facing slopes of hills or along cracks and crevices in rocks (Viljoen et al., 2002). Traditionally, the whole plant is used for colds, respiratory ailments, nosebleeds, and fainting, while the smoke from the burnt dried leaves is inhaled for asthma and chest pain or channeled into the vagina to treat vaginal infections and uterine pains (Viljoen et al., 2002). Previous studies have given credence to the in vitro antioxidative, antidiabetic, and antibacterial properties of the leaf and shoot extracts as well as the essential oils of *M. flabellifolius* (Kwape, Majinda, & Chaturvedi, 2016; Moore et al., 2005; Viljoen et al., 2002), which may be influenced by its phytochemical constituents.

3,4,5-Tri-O-galloylquinic acid have been previously isolated from the plant's leaf (Moore et al., 2005). Qualitative analysis of the leaf decoction showed the presence of tannins (Bhebhe et al., 2015), while that of the ethanol and hydroethanol extracts of the leaf showed the presence of tannins, flavonoids, alkaloids, terpenoids, saponins, glycosides, and phytosterols (Ajao & Ashafa, 2017; Dzomba, Gwatidzo, Mtwazi, & Mupa, 2016; Kwape et al., 2016). Furthermore, LC-MS analysis of the leaf hot-water extract identified catechin, p-coumaric acid, caffeic acid, epicatechin, and chlorogenic acid, with epicatechin being the most abundant (Thomford et al., 2016). On the other hand, GCMS analysis of the leaf essential oil extract showed, in ascending order, limonene, cis-p-menth-1-(7)-8-diene-2-ol, rans-p-menth-1-(7)-8-diene-2-ol, pinocarvone, and trans-pinocarveol as the most abundant compounds (Viljoen et al., 2002).

Bhebhe et al. (2015) reported the DPPH radical scavenging, ferric reducing and phospholipid anti-peroxidative activities of the leaf decoctions. The ethanol and hydroethanol extracts of the leaf were, also, reported to show α-glucosidase and α-amylase inhibition, as well as in vitro radicals scavenging and ferric reducing antioxidant activities (Ajao & Ashafa, 2017; Dzomba et al., 2016; Kwape et al., 2016). On the other hand, the leaf essential oil showed antimicrobial effects against several microbial strains (Viljoen et al., 2002), while the leaf decoction showed CYP2B6 inhibitory activity, which was partly attributed to the identified polyphenols (Thomford et al., 2016). *M. flabellifolius* leaf has been locally used as a flavored medicinal tea, perhaps due to the presence of camphor and eucalyptol present in the leaf essential oils (SANBI, 2013). Presently, there is little or no scientific data available on the proximate nutritional composition and antioxidative potential of the leaf decoction of this plant, which has promising potential of commercialization as a herbal tea.

The present study was conducted to provide useful scientific information on the proximate and nutritional composition of *M. flabellifolius* hot water extract, as well as its protective potential against oxidative hepatic cell injury.

2 | MATERIALS AND METHODS

2.1 | Plant collection and extraction

M. flabellifolius (Welw.) plant material was collected from the wild in Mokgola, North West Province, South Africa (GPS coordinate: 25.318441° S, 23.11683° E) by M. G. Matsabisa. The plant sample

was deposited and authenticated at the Geo Potts herbarium (BLFU), University of the Free State (specimen voucher number: BLFU-MGM0011). The leaves were washed, dried at room temperature, and pulverized into a fine powder. The powdered leaves (100 g) were extracted in 500 ml of boiling distilled water for 10 min with agitation. After cooling, the mixture was filtered with a Whatman filter (Whatman, England) and the filtrate was freeze-dried and stored at −20°C. The crude extracts were reconstituted in distilled water to prepare the various concentrations used for the phytochemical characterization, nutritional composition, and the in vitro antioxidant assays.

2.2 | Nutritional analysis

The nutritional composition MLTE was done by the Agricultural Research Council (ARC), South Africa (ARC-Irene Analytical Services, Pretoria South Africa; Facility Accreditation Number: T0063). The analyses for the nutritional content of *M. flabellifolius* were based on SANAS (South African National Accreditation System) accredited methods. Vitamin A (Hulshof, 2002), E (Manz & Philipp, 1981), B1 (Sims & Shoemaker, 1993), B2 (Sims & Shoemaker, 1993), and C (Dodson, Young, & Soliman, 1992), where analyzed using previously reported liquid chromatographic techniques.

2.3 | HPLC identification of polyphenols

The tea extract was analyzed using HPLC to identify phenolic compounds present. HPLC coupled with diode array detector (DAD) analysis was performed using an Agilent 1100 series (Agilent, Waldbronn, Germany) instrument equipped with photodiode array, autosampler, column thermostat, and degasser. The extract and phenolic standards were analyzed at a concentration of 5 mg/ml. A Phenomenex: Luna 5 µm C_{18} (2) (150 × 4.6 mm; 5 µm particle size) column was used as the stationary phase. Water containing 0.01% of formic acid (A) and acetonitrile (B) was served as mobile phase at a flow rate of 1 ml/min. Gradient elution was applied as follows: Initial ratio 95% A: 5% B, keeping for 10 min, changed to 90% A: 10% B in 10 min, changed to 70% A: 30% B in 10 min, to 50% A: 50% B in 10 min, maintaining for 0.5 min and back to initial ratio in 0.5 min. The temperature was set to 30°C. The injection volume was 20 µl and chromatograms were recorded at wavelength of 254 nm.

2.4 | Measurement of phenol, flavonol, and flavanol contents

To measure the phenol content of MLTE, the method reported by Chukwuma, Islam, and Amonsou (2018) was used. The flavonoid (flavonol + flavanol) content of the tea extract was estimated as flavonol and flavonoid contents. To measure the flavonol and flavanol contents, the methods of Lee, Shukla, Kim, and Kim (2015) and Treutter (1989) were used, respectively. Extract was tested at a concentration of 120 µg/ml and the phenol, flavonol, and flavanol contents of the sample was determined from standard curves (10–240 µg/ml) of gallic acid for phenol content (mg/g GAE) or quercetin for flavonol content (mg/g QE) or catechin for flavanol content (mg/g CE) using the following formula:

$$\text{Phenol content}\left(\text{mg/g GAE}\right) = \frac{C \times SV}{M} \quad (1)$$

where C is the concentration (µm) extrapolated from standard curve, SV is the sample volume (ml), and M is the mass (g) of the sample in SV (ml) of the sample solution.

2.5 | Measurement of in vitro antioxidant capacity

Previous methods were adopted with slight modifications to measure the antioxidant capacity of MLTE using four in vitro models: (a) ferric reducing antioxidant power (FRAP) measured as gallic acid equivalent (Chukwuma et al., 2018); (b) 2,2-azinobis (3-ethylbenzothiazoline 6-sulfonate) (ABTS) radical antioxidant capacity (TAC) measured as Trolox equivalent (Zhang et al., 2014); and (c) oxygen radical absorbance capacity (ORAC) measured as Trolox equivalent (Wu et al., 2004) and (d) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging (Sanni et al., 2019).

2.6 | Measurement of protective effects against induced oxidative damage in Chang liver cells

This assay was modified from a previous method (Sanni et al., 2019). Cultured human Chang liver cells (EMEM supplemented with 10% FBS) at 90% confluence, were pretreated with different concentrations (15–120 µg/ml) of MLTE or standards (ascorbic acid and gallic acid) before inducing oxidative stress with a 7 mM $FeSO_4$. After 30-min incubation, the level of lipid peroxidation and reduced glutathione was measured in the supernatants (Mchunu et al., 2019; Sanni et al., 2019) and compared to the normal cells and induced cells without treatment.

2.7 | Cell proliferation assay

The effect of MLTE on cell proliferation done the Chang liver cells using standard MTT assay. The extract and the positive controls (doxorubicin and verapamil) were tested at concentrations of 31.25–500 µg/ml and 6.25–100 µg/ml, respectively. The results were presented as the mean ± *SD* the percentage inhibition of biological repeats.

2.8 | Statistical analysis

Results were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple range post hoc tests (IBM, SPSS, version 23) and p values less than .05 were considered significant when comparing groups.

3 | RESULTS AND DISCUSSION

The antioxidant benefits of herbal teas have strongly influenced their consumption, although most herbal teas in literature lack scientific

data on their proximate and nutritional composition. *M. flabellifolius* (Welw.) is a widely used traditional herbal tea with potentials for commercialization due to its pleasant flavor. Its in vitro pharmacological properties have been reported (Bhebhe et al., 2015), without any scientific information on its nutritional composition. The present study is the first to report the nutritional composition and hepatic antioxidative activities of its leaf decoction.

3.1 | Yield, polyphenols content, and HPLC Identification of polyphenols

Extraction yielded 14.74% of extract. The flavonol, flavanol, and phenol contents were 14.84 ± 0.64 mg/g QE, 1.47 ± 0.08 mg/g CE, and 82.26 ± 5.30 mg/g GAE, respectively (Table 3). Gallic acid, caffeic acid, ferulic acid, methyl gallate, and (-)-epicatechin were identified in MLTE at retention times 4.478, 16.025, 19.955, 13.435, and 16.911 min, with percentage peak areas of 26.6%, 3.9%, 15.2%, 3.7%, and 3.1%, respectively (Table 1). The major peaks for the *M. flabellifolius* tea extract corresponded with gallic acid and ferulic acid with lesser amounts of the other polyphenols.

3.2 | Nutritional composition

MLTE has an appreciable nutritional value, composing of proteins, carbohydrates, lipids, dietary fibers, vitamins, and minerals (Table 2). The low moisture content (9.6%) in the tea extract (Table 2) confirms suggests its capacity for long-term storage against microbial invasion. The amount of dietary fiber (29.6%) and ash content registered in MLTE is lower than that of mamaki tea leaves but considerably higher than reported amounts in commercial Lipton tea (*Camellia sinensis*) (Kartika, Shido, Nakamoto, Li, & Iwaoka, 2011), which can contribute to the daily needs of fiber to reduce the risk of diabesity, cancer, and cardiovascular diseases without causing bowel irritation (Theuwissen & Mensink, 2008). The ash content of teas has been reported to be inversely proportional to the total mineral elements and the quality of the tea during storage (Kartika et al., 2011). Consequently, the low level of ash content (7.9%) detected in MLTE suggests that it may be a good source of minerals. The ash content is also within the recommended range (5.54%) to maintain the quality of tea during storage (Qasim et al., 2017). The crude protein composition of MLTE was comparable with those recorded in the green teas and *Lippia multiflora* herbal tea grown in Abidjan (Christine, Albert, & Séraphin, 2017) and within the recommended daily need by the Dietary Reference Intake (DRI, 2000). Furthermore, the low percentage of fat, cholesterol, and salt in MLTE (Table 2) suggests its potential to circumvent the risk of obesity and high blood pressure (Kartika et al., 2011). The high composition of carbohydrate (73%) in MLTE correlates with its computed energy value (475 kJ/100 g) (Table 2), which may serve as a veritable source for a high-calorie nutrition program.

TABLE 1 Retention times and percentage peak areas of HPLC-identified polyphenols in MLTE

Polyphenols	Parameters	Tea extract
Gallic acid (RT: 4.458 min)	RT (min)	4.478
	DFS (min)	+0.02
	% Peak area	26.6260%
Caffeic acid (RT: 15.897 min)	RT (min)	16.025
	DFS (min)	+0.128
	% Peak area	3.9368%
Ferulic acid (RT: 19.937 min)	RT (min)	19.955
	DFS (min)	+0.018
	% Peak area	15.2357%
Methyl gallate (RT: 13.81 min)	RT (min)	13.435
	DFS (min)	−0.375
	% Peak area	3.7159%
(-)-Epicatechin (RT: 16.83 min)	RT (min)	16.911
	DFS (min)	+0.081
	% Peak area	3.0900%

Note: RT, peak retention time; DFS, the difference between the peak retention time of the standard polyphenol and the peak retention time of that shown in the HPLC chromatogram of MLTE.

The composition of sugars (glucose, sucrose, fructose) detected in MLTE can contribute to the recommended carbohydrate daily allowance (130 g/day) without risk of hyperglycemia (Murphy & Johnson, 2003). Excluding glutamine and asparagine, 18 amino acids, including essential and nonessential amino acids, were identified (Table 2). Glutamic acid (0.38 g/100 g), aspartic acid (0.32 g/100 g), leucine (0.29 g/100 g), and lysine (0.29 g/100 g) were the most abundant, whereas methionine, HO-proline, cysteine, and tryptophan had the lowest composition (0.01–0.07 g/100 g). Other identified amino acids were detected in relatively fair amounts (0.12–0.24 g/100 g). Lysine and leucine have been reported as the essential amino acids with the highest daily recommended intakes for infants, children of 2 and 10–12 years and adults (RDA, 1989), which suggests the potent nutritional benefit of MLTE. Based on the amino acids composition, it is apparent that consumption of *M. flabellifolius* herbal tea has a great potential to contribute to the daily requirement of amino acids for the normal functioning of the human body (Table 2). The fatty acid profiling showed that MLTE contained more of unsaturated fatty acids, including the omega 3, 6, and 9 fatty acids (Table 2), which are useful in the management of cardiovascular disorders and related diseases. It has been reported that palmitic acid increases the blood cholesterol, while oleic acid decreases blood cholesterol level and stearic acid has no effect (Peña et al., 2009). Although these fatty acids (FAs) are the most abundant in MLTE (Table 2), the ratio of oleic acid and stearic acid to palmitic acid [(C18:0 + C18:1)/C16:0] was 2.83, which suggests the potential usefulness of *M. flabellifolius* herbal tea in controlling the blood cholesterol level.

The amount of dietary minerals supply per cup of tea consumed was computed from Table 2 using the methods of Olivier, Symington, Jonker, Rampedi, and Van Eeden (2012). Based on this computation, MLTE showed lesser amounts in calcium (Ca), phosphorus (P), magnesium (Mg), iron (Fe), and potassium (K) (11.72, 2.31, 2.101, 0.76, and 6.27 mg/250 ml, respectively) compared to rooibos tea, but higher amounts compared to *Camellia sinensis* and *Lippia multiflora* herbal

TABLE 2 Nutritional composition of MLTE

Nutrient	Composition	Unit
Proximate		
Dry matter	90.40	%
Moisture	9.60	%
Ash	7.86	%
Total dietary fiber	29.57	%
Protein	5.85	%
Fat	2.86	%
Total nonstructural carbohydrates	15.87	%
Water-soluble carbohydrates (solid)	7.49	%
Carbohydrates (calculated)	73.83	%
Starch	ND	%
Salt	0.06	%
Cholesterol	3	mg/100 g
Energy (calculated)	475	kJ/100 g
Gross energy	18.60	MJ/kg
Sugars		
Glucose	2.52	g/100 g
Fructose	0.93	g/100 g
Sucrose	2.40	g/100 g
Maltose	ND	g/100 g
Lactose	ND	g/100 g
Rafinose	2.49	g/100 g
Stachiose	2.18	g/100 g
Saturated fatty acids		
C8:0 (Caprylic acid)	0.004	%
C10:0 (Capric acid)	0.013	%
C12:0 (Lauric acid)	0.037	%
C13:0 (Tridecylic acid)	0.001	%
C14:0 (Myristic acid)	0.066	%
C15:0 (Pentadecyclic acid)	0.006	%
C16:0 (Palmitic acid)	0.500	%
C17:0 (Margaric acid)	0.013	%
C18:0 (Stearic acid)	0.407	%
C20:0 (Arachidic acid)	0.044	%
C21:0 (Heneicosylic acid)	0.004	%
C22:0 (Behenic acid)	0.077	%
C23:0 (Tricosylic acid)	0.005	%
C24:0 (Lignoceric acid)	0.019	%
Total saturated fatty acid	1.196	%
Mono- and polyunsaturated fatty acids		
C14:1 (Myristoleic acid)	0.004	%
C15:1 (*cis*-10) (Pentadecenoic acid, *cis*-10)	0.001	%
C16:1 (Palmitoleic acid)	0.010	%
C17:1 (Heptadecenoic acid, *cis*-10)	0.004	%
C20:1 (Eicosenoic acid)	0.009	%

(Continues)

TABLE 2 (Continued)

Nutrient	Composition	Unit
C20:2 (Eicosadienoic acid)	0.002	%
C22:2 (Docosadienoic acid)	0.008	%
Total monounsaturated fatty acids	1.035	%
Total polyunsaturated fatty acids	0.608	%
Total *trans* fatty acids	0.020	%
Total *cis* fatty acids	1.450	%
Omega 3, 6, and 9 unsaturated fatty acid		
C18:3 (*n*-3) (α-Linolenic acid)	0.141	%
C20:5 (*n*-3) (Eicosapentaenoic acid)	0.006	%
C22:6 (*n*-3) (Docosahexaenoic acid)	0.004	%
C18:2 *cis* (*n*-6) (Linoleic acid)	0.442	%
C18:2 *trans* (*n*-6) (Linoelaidic acid)	0.002	%
C20:3 (*n*-6) (Dihomo-gamma-linolenic acid)	0.001	%
C20:4 (*n*-6) (Arachidonic acid)	0.004	%
C18:1 *cis* (*n*-9) (Oleic acid)	1.007	%
C18:1 *trans* (*n*-9) (Elaidic acid)	0.018	%
Total Omega 3 fatty acids	0.151	%
Total Omega 6 fatty acids	0.450	%
Total Omega 9 fatty acids	1.025	%
Amino acid		
Cysteine	0.01	g/100 g
Tryptophan	0.01	g/100 g
Arginine	0.24	g/100 g
Serine	0.19	g/100 g
Aspartic acid	0.32	g/100 g
Glutamic acid	0.38	g/100 g
Glycine	0.20	g/100 g
Threonine	0.20	g/100 g
Alanine	0.22	g/100 g
Tyrosine	0.13	g/100 g
Proline	0.21	g/100 g
HO-Proline	0.07	g/100 g
Methionine	0.06	g/100 g
Valine	0.22	g/100 g
Phenylalanine	0.21	g/100 g
Isoleucine	0.20	g/100 g
Leucine	0.29	g/100 g
Histidine	0.12	g/100 g
Lysine	0.29	g/100 g
Vitamins		
Vit A	0.05	mg/100 g
Vit B1	ND	mg/100 g
Vit B2	0.13	mg/100 g
Vit C	ND	mg/100 g
Vit E	3.77	mg/100 g

(Continues)

TABLE 2 (Continued)

Nutrient	Composition	Unit
Major minerals		
Calcium	468.84	mg/100 g
Phosphorous	92.44	mg/100 g
Magnesium	84.02	mg/100 g
Potassium	250.58	mg/100 g
Sodium	18.01	mg/100 g
Chloride	23.04	mg/100 g
Trace minerals		
Chromium	3.53	mg/kg
Copper	4.28	mg/kg
Fluoride	31.92	mg/100 g
Iron	302.56	mg/kg
Manganese	198.43	mg/kg
Zinc	20.94	mg/kg
Molybdenum	0.10	mg/kg

Note: ND, not detected.

TABLE 3 Total phenol content, total flavonoid content, and antioxidant capacity of MLTE

Parameter	Composition/activity	Unit
Total phenol content	82.26 ± 5.30	mg/g GAE
Total flavonoid content		
Total flavonol content	14.84 ± 0.64	mg/g QE
Total flavanol content	1.47 ± 0.08	mg/g CE
Ferric reducing antioxidant power (FRAP)	546.89 ± 13.41	μmol/g AAE
ABTS radical antioxidant capacity (TAC)	448.97 ± 43.50	μmol/g TE
Oxygen radical absorbance capacity (ORAC)	1,780 ± 90.00	μmol/g TE

Note: Data present as ± *SD* of triplicate.
Abbreviations: GAE, gallic acid equivalent; QE, quercetin equivalent; CE, (+)-catechin equivalent; AAE, ascorbic acid equivalent; TE, trolox equivalent.

green teas (McKenzie, Jurado, & De Pablos, 2010; Morton, 1983). Nevertheless, MLTE was rich in calcium (468.84 mg/100 g), potassium (250.58 mg/100 g), phosphorus (92.44 mg/100 g), magnesium (84.02 mg/100 g), fluoride (31.92 mg/100 g), iron (302.56 mg/kg), manganese (198.43 mg/kg), and zinc (20.94 mg/kg) among the mineral elements identified. The calcium and other minerals contained in MLTE can contribute to the daily recommended calcium (900 mg/day) and other mineral requirements needed for bone growth, maintenance of blood pressure, prevention of blood coagulation, and other metabolic functions (Alais, Linden, & Miclo, 2008). Based on the macro- and micromineral compositions (Table 2), *M. flabellifolius* consumption may compensate for the subnormal levels of some of these minerals in people living with diabetes, anemia, immune, and myocardial dysfunction, due to their role in several metabolic functions (Alais et al., 2008).

The amount of vitamin E in MLTE was more than 25 times that of vitamin A and B2 (Table 2), although it is lesser than the dose (15 mg/day) recommended as the dietary reference intake (DRI, 2000). Nevertheless, the reported protective role of vitamin E against cellular oxidative damage in several diseases (Rizvi et al., 2014) suggests that *M. flabellifolius* herbal tea may be useful in the management of oxidative stress-associated complications in several diseases.

3.3 | In vitro antioxidant capacity

The antioxidative potency of *M. flabellifolius* may also be a reflection of its phytochemistry. The phenol content (82.26 ± 5.30 mg/g GAE) observed in this study (Table 3) is higher than that reported by Bhebhe et al. (2015) on *M. flabelifolius* cultivated in Zimbabwe (47.5 ± 0.33 mg/g GAE) and commercial rooibos tea (66.9 ± 0.83 mg/g GAE), which suggests that MLTE may be a rich source of polyphenol, known to exerts a wide range of pharmacological and antioxidant properties (Sanni et al., 2019). Gallic acid appeared to be the most abundant among the identified polyphenols based on the HPLC chemical profile (Table 1). The versatile antioxidant properties of gallic acid and its therapeutic applications is known, suggesting that *M. flabellifolius* herbal tea could be a promising antioxidant beverage. MLTE showed ferric reducing antioxidant (9,546.89 ± 13.41 μmol/g AAE), oxygen radical absorbance (1,780 ± 90.00 μmol/g TE), and ABTS radical quenching (448.97 ± 43.50 μmol/g TE) activities

TABLE 4 IC_{50} values for antioxidant properties of MLTE

Teas and standards	IC_{50} (μg/ml)		
	DPPH scavenging activity	Inhibition of lipid peroxidation in hepatocytes	Inhibition of GSH depletion in hepatocytes
Tea extract	62.99 ± 15.83^{e}	113.11 ± 4^{b}	70.49 ± 24.39^{a}
Gallic acid	0.04 ± 0.02^{a}	76.91 ± 19.57^{a}	77.51 ± 29.64^{a}
Ascorbic acid	0.26 ± 0.08^{b}	66.93 ± 24.98^{a}	65.39 ± 3.76^{a}
Quercetin	33.11 ± 4.92^{d}	ND	ND
Trolox	13.49 ± 0.31^{c}	ND	ND

Note: ND means not determined. Data presented as the mean ± *SD* of replicate values. Different alphabets signify significant difference ($p < .05$) between treatments for a given parameter.

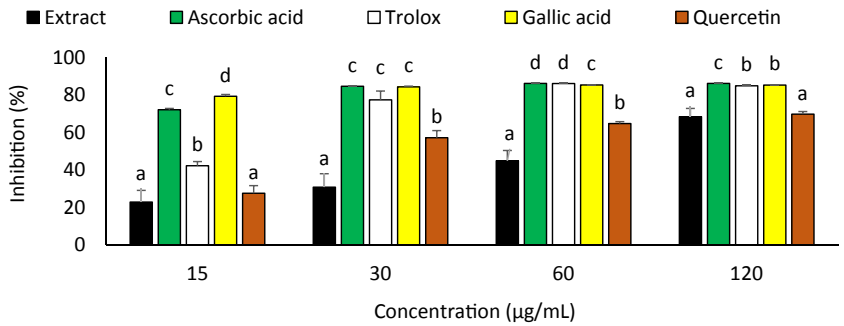

FIGURE 1 DPPH scavenging ability of MLTE. Data presented as the mean ± *SD* of replicate values. Different alphabets signify significant difference ($p < .05$) between treatments for a given concentration

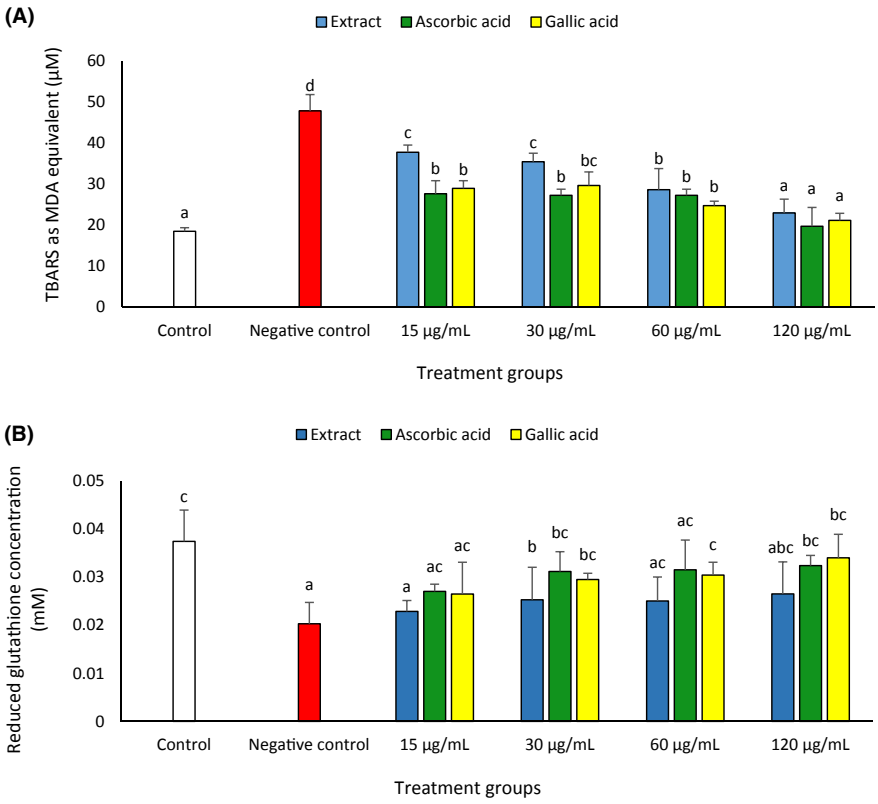

FIGURE 2 Lipid peroxidation and (A) GSH levels (B) in in Chang liver cells treated with MLTE. Data presented as the mean ± *SD* values of biological repeats. Different alphabets signify significant difference ($p < .05$) between treatments for a given concentration and the controls

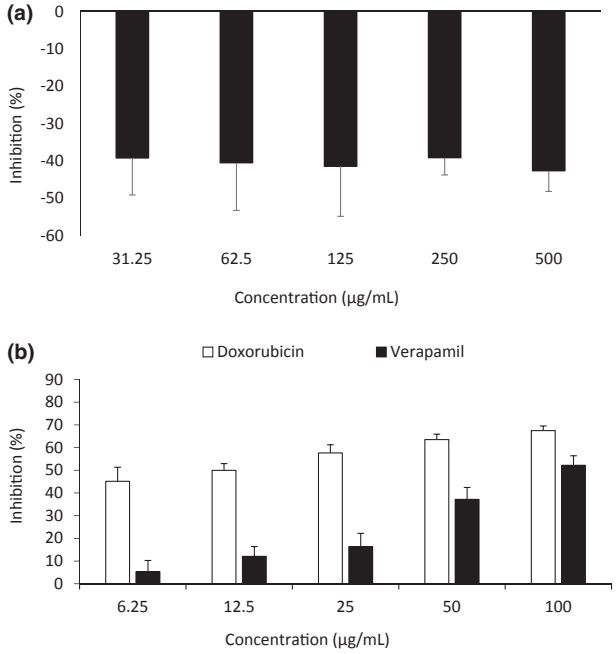

FIGURE 3 Chang liver cell proliferation inhibition (%) of (a) MLTE and (b) standard drugs, doxorubicin and verapamil. Data presented as mean ± *SD* values of biological repeats

(Table 3). The inhibitory concentration obtained for MLTE extract against DPPH radical (IC_{50} = 62.99 ± 15.83 µg/ml) (Table 4 and Figure 1) was more potent than those reported for South African herbal teas, namely *Aspalathus linearis* (rooibos), Cyclopia spp, and *Camelia sinensis* teas, as well as the value recorded for *M. flabelifolius*, cultivated in Zimbabwe (Bhebhe et al., 2015; Joubert, Gelderblom, Louw, & De Beer, 2008), which may be attributed to the rich polyphenol composition including gallic acid (Tables 1 and 3).

3.4 | Protective effect against oxidative stress in Chang liver cells

Induction of oxidative stress in Chang liver cells leads to a significant ($p < .05$) increase (61.4%) in lipid peroxidation ($p < .05$) and a significant ($p < .05$) decrease (45.8%) in reduced glutathione (GSH) level (Figure 2). Pretreatment with MLTE dose dependently suppressed the oxidative stress-induced by lipid peroxidation (IC_{50} = 113.11 ± 4 µg/ml) and GSH reduction (IC_{50} = 70.49 ± 24.39 µg/ml) in the hepatocytes (Figure 2 and Table 4). At 60 and 120 µg/ml, the protective effects of MLTE on lipid peroxidation were comparable to ascorbic and gallic acids (Figure 2A). Its inhibitory effect on GSH depletion did not differ significantly from that of ascorbic (IC_{50} = 65.39 ± 3.76 µg/ml) and gallic acids (IC_{50} = 77.51 ± 29.64 µg/ml) (Figure 2B and Table 4). These results suggest a remarkable antioxidant capacity of MLTE against hepatic oxidative damage. This noteworthy effect of the tea extract may be linked to the high composition of vitamin E (Table 2), which has been reported to protect against cellular oxidative damage and lipid peroxidation in several diseases by trapping peroxyl radicals ($ROO^{\bullet}$) (Rizvi et al., 2014).

Moreover, although doxorubicin and verapamil showed dose-dependent inhibitions (IC_{50} = 20.95 ± 0.25 and 44.31 ± 2.11 µg/ml, respectively) (Figure 3b) on the growth and proliferation of Chang liver cells, MLTE showed a proliferative rather than an inhibitory on the liver cells (Figure 3a), even at a concentration (500 µg/ml) higher than the cellular antioxidant IC_{50} concentrations (113.11 ± 4 and 70.49 ± 24.39 µg/ml) (Table 4). These data support the safety of *M. flabellifolius* herbal tea and its possible beneficial effects in wound healing and cell regeneration or rejuvination processes.

4 | CONCLUSION

M. flabellifolius herbal tea has a nutritional value. The nutritional data of this herbal tea provided in this study can inform the dietary and nutritional use of the tea for optimum benefits. The leaf decoction showed an appreciable antioxidant capacity, in vitro and in hepatocytes, which may be attributed to the phenolics and vitamin E composition. This study is a good preliminary scientific validation of the potential use of *M. flabellifolius* herbal tea as an antioxidant beverage with good nutritional value.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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