Journal of Materials and Environmental Science
ISSN : 2028-2508
e-ISSN : 2737-890X
CODEN : JMESCN

J. Mater. Environ. Sci., 2023, Volume 14, Issue 11, Page 1196-1203

http://www.jmaterenvironsci.com

Phytochemicals, Chemical Composition and Antioxidants profile of the crude extracts and essential oil of *Leucas Martinicensis*

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Received *29 Sept 2023,*
Revised *05 Nov 2023,*
Accepted *09 Nov 2023*

Keywords:
- ✓ *Scavenging Activities;*
- ✓ *Free radicals*
- ✓ *DPPH;*
- ✓ *FRAP;*
- ✓ *GC_MS;*
- ✓ *Extracts*

Citation*: Ali M.N., Balarabe A., Tanimu F.B., Anchau H.G., Jocthan M.G., Suleiman A.N., Yelwa J.M. (2023) Phytochemicals, Chemical Composition and Antioxidants profile of the crude extracts and essential oil of Leucas Martinicensis, J. Mater. Environ. Sci., 14(11), 1196-1203*

Abstract: This study investigated the phytochemicals, chemical composition and antioxidant profile of the crude extracts and essential oil derived from *Leucas Martinicensis*. The plant material was collected, dried, and subjected to extraction using methanol. The essential oil was obtained by hydrodistillation using a Clevenger-type apparatus. The phytochemical analysis of the crude extracts and essential oil was performed using standard qualitative and quantitative methods. The results revealed the presence of diverse phytochemical constituents such as flavonoids, alkaloids, tannins, saponins, and terpenoids in the crude extracts and essential oil of *Leucas Martinicensis*. Additionally, the essential oil was found to be rich in various volatile compounds, such as 2-propenoic acid 3 [(phenylmethyl) thiol] (E) (71.21%) and squalene (3.34%). Furthermore, the antioxidant potential of the crude extracts and essential oil was evaluated using various antioxidant assays, including DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay and ferric reducing antioxidant power (FRAP) assay. The results demonstrated strong antioxidant activity in both the crude extracts and essential oil, with notable variations among different solvents and concentrations. The observed antioxidant potential may be attributed to the presence of phenolic compounds and other bioactive constituents. This study highlights the phytochemical diversity and antioxidant profile of the crude extracts and essential oil derived from *Leucas Martinicensis*. The presence of various phytochemicals suggests its potential as a natural source of bioactive compounds. Moreover, the significant antioxidant activity exhibited by the extracts and essential oil indicates their possible application in the pharmaceutical and food industries.

1. Introduction

Leucas martinicensis, commonly known as "wild basil" or "cow basil," is a medicinal plant belonging to the Lamiaceae family. It is widely distributed in tropical and subtropical regions, including parts of Asia, Africa, and the Caribbean. *Leucas martinicensis* has a long history of traditional use in various folk medicinal systems due to its reported therapeutic properties. The genus Leucas encompasses a diverse group of plants that are known for their bioactive compounds and pharmacological potential (Musa, 2017). Among these, *Leucas martinicensis* has gained attention as a valuable medicinal plant due to its promising chemical constituents and associated biological activities.

Exploring the phytochemical composition and antioxidant profile of *Leucas martinicensis* can provide valuable insights into its therapeutic potential and contribute to its utilization in various health-related applications (Mohammed *et al*., 2012).

Phytochemicals are naturally occurring biologically active compounds found in plants. They are responsible for the distinct medicinal properties exhibited by medicinal plants (Musa, 2017; El Ouadi *et al.*, 2017). The main phytochemical components, present in medicinal plants are tannins, alkaloids, saponins, cardiac glycosides, steroids, terpenoids, flavonoids, phlobatannins, anthraquinones, and reducing sugars (Haddou *et al.*, 2023; El Ouariachi *et al.*, 2011). These natural chemical compounds play a critical role in their survival and proper function. These chemical components not only protect plants from competitors, pathogens, or predators but also control the growth along with regulating the pollination, fertilization, and the rhizosphere environment (Molyneux *et al*., 2007; Rabizadeh *et al*., 2022). *Leucas martinicensis* is known to contain various classes of phytochemicals, including alkaloids, flavonoids, terpenoids, phenolic compounds, and saponins. These phytochemicals have been associated with a wide range of biological activities such as antioxidant, antimicrobial, anti-inflammatory, and anticancer effects (Hina *et al*., 2011; Selles *et al.*, 2013). Understanding the chemical composition of *Leucas martinicensis* is crucial for harnessing its therapeutic potential (Uba *et al*., 2021). The chemical constituents of this plant can vary significantly depending on factors such as geographical location, climatic conditions, and plant growth stage. Various analytical techniques, such as gas chromatography-mass spectrometry (GC-MS), high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR) spectroscopy, are commonly employed to identify and quantify the specific compounds present in the plant (Joshi, 2017; Lamia *et al*., 2013).

Antioxidants play a vital role in maintaining cellular health by neutralizing harmful free radicals and reducing oxidative stress (Yahaya *et al*., 2018). *Leucas martinicensis* has been reported to possess antioxidant activity, which can be attributed to its phytochemical composition. Antioxidants protect the body against oxidative damage, which is implicated in several chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer. Evaluating the antioxidant profile of *Leucas martinicensis*, including the determination of its antioxidant capacity and identification of specific antioxidants present, can provide valuable insights into its potential therapeutic applications (Maria, 2010).

In this study, investigated the phytochemicals, chemical composition, and antioxidant profile of the crude extracts and essential oil of *Leucas martinicensis*. Through a comprehensive analysis, including qualitative and quantitative assessments, we identified the major phytochemical constituents, determine the chemical composition of the extracts, and evaluated their antioxidant activity. This research will contribute to the scientific understanding of *Leucas martinicensis* and provide a foundation for further exploration of its therapeutic potential.

2. Methodology

2.2 Experiments

Leucas Martinicensis plant material was collected from Yola North Local Government Area of Adamawa State. Methanol was selected for the extraction of bioactive compounds from Leucas Martinicensis. The essential oil of Leucas Martinicensis was obtained by steam distillation using modified steam distiller (as modified by Runde *et al*., (2015)) according the British pharmacopoeia (BP) method. The phytochemical analysis of the crude extracts and essential oil was performed using standard qualitative methods as described by Kubmarawa *et al.* (2013) & Kadda *et al.* (2022).

The chemical composition of the essential oil was analyzed using gas chromatography-mass spectrometry (J and W Scientific). The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out using a method describe by Yahaya *et al*. (2018), H_2O_2 was performed according to Keshari *et al*., (2017) and FRAP was carried out according to method described by Anjali Soni *et al*., (2013) with slight modification.

Scheme 1. *Leucas Martinicensis* studied

3. Results and Discussion

3.1 Phytochemical Analysis of Leucas Martinicensis

In this study, we investigated the phytochemical composition, chemical composition, and antioxidant activity of *Leucas Martinicensis*. The results revealed a rich phytochemical profile in the crude extracts, including flavonoids, alkaloids, glycosides, essential oils, tannins, saponins, and terpenoids (**Table 1**). These phytochemicals are known for their various biological activities and potential health benefits Judzentiene *et al*., 2023).

Table 1. Qualitative phytochemical analysis

S/No	Phytochemicals	*Leucas Martinicensis*
1	Saponins	+++
2	Tannins	+
3	Flavonoid	++
4	Alkaloid	+++
5	Essential oils	++
6	Glycosides	++
7	Phenols	ND
8	Resins	ND

3.2 Chemical Composition of Essential Oil from Leucas martinicensis

The GC-MS analysis of the essential oil of *Leucas Martinicensis* identified several volatile compounds including 7-isopropyl-1-,4a trimethyl-1,2,3,4,4a,9,10,10a octahydro phenanthrene (1.26%) 10-undecyn-1-ol (1.11%), benzenethanamine, alpha 2,6trimethyl (.+/-.)(1.48%), 2-propenoic acid 3 [(phenylmethyl) thiol] (E) (71.21%), trans-cinamyl bromide (1.20%) squalene (3.34%), 1,2-propane diol,3-benzyloxy 1,2-diacetyl (1.88%), were found to be present in significant amounts which

contributed to the aroma and potential therapeutic properties of the plant (Uba *et al*., 2021). As presented in **Table 2,** these compounds are mainly sesquiterpenic hydrocarbon (84.39%), the most prominent being: β-caryophyllene (43.86%); α-humuene (19.93%); germacrene D (7.09%); caryophyllene oxide (2.4%) and β-eudesmol (1.27%). In the essential oil sample of C. Zambesicus, thirty-two (32) components were detected and identified. This oil was composed mainly of monotrepenes (62.14%), but the main constituents were two sesquiterpenic hydrocarbon: β-Elemol (13.23%) and β-Eudesmol (10.52%). Sesquiterpenic hydrocarbon represented only (33.52%) of this oil. These results are different from those reported in Benin (Senatore *et al*., 2013). These volatile compounds are known for their various pharmacological activities, such as antimicrobial and anti-inflammatory effects (Ali *et al*., 2021). The presence of these compounds further highlights the potential therapeutic value of the essential oil (Ogwuche and Edema, 2020).

3.3 Antioxidant Assessment

The antioxidant properties of the extracts were determined with reference to ascorbic acid (vitamin C), which is considered a natural oxidant. The results revealed that all the extracts exhibited anti-radical effects as a function of time (Uba *et al*., 2021; Khadhim *et al*., 2016). An increase or a decrease in the percentage of inhibition was observed every 3 minutes. Similarly, the rate of inhibition also varies as a function of time. This trend was also observed in ascorbic acid (vitamin C), which is the reference molecule with a radical-activity value of 96.617%.

Table 2. Chemical constituents of essential oil from *Leucas Martinicensis*

Compounds	Molecular Formula	Molecular Weight	Retention Time (Min)	Area (%)
Benzene, (5-bromopentyl)-	$C_{11}H_{15}Br$	226	12.992	2.62
3-(Benzylthio)acrylic acid, methyl ester	$C_{11}H_{12}O_2S$	208	13.690	**9.28**
7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	$C_{20}H_{30}$	270	17.060	1.26
10-Undecyn-1-ol	$C_{11}H_{20}O$	168	17.403	1.11
Benzeneethanamine,.alpha.,2,6-trimethyl-, (.+/-.)-	$C_{11}H_{17}N$	163	19.881	1.48
2-Propenoic acid, 3-[(phenylmethyl)thio]-,(E)-	$C_{10}H_{10}O_2S$	194	20.482	**71.21**
trans-Cinnamyl bromide	C_9H_9Br	196	21.924	1.20
Squalene	$C_{30}H_{50}$	410	23.154	3.34
1,2-Propanediol, 3-benzyloxy-1,2-diacetyl-	$C_{14}H_{18}O_5$	266	26.307	1.88

The evaluation of the antioxidant activity demonstrated significant scavenging potential in the crude extracts and essential oil (**Figures 1, 2 & 3**). The observed IC50 values indicate effective free radical scavenging ability, suggesting that *Leucas Martinicensis* possesses antioxidant compounds that can protect against oxidative stress-related diseases (Madouni *et al*., 2021). The hydrogen peroxide scavenging activity of *Leucas Martinicensis* has increased with increased concentration of both the extract and the essential oil. Moreover, the FRAP assay results showed a high ferric reducing antioxidant power in the extracts and essential oil. This indicates their capacity to reduce ferric ions to ferrous ions, reflecting their ability to act as electron donors and counteract oxidative processes

(Abegaz and Dagne, 2012). The observed antioxidant activity can be attributed to the presence of phenolic compounds, flavonoids, and other bioactive constituents in *Leucas Martinicensis* (**Table 3**).

Table 3. Results of antioxidant activities of Crude Extract, Essential oil and Ascorbic acid (Control)

	Crude Extract			Essential oil			Ascorbic acid (Control)		
Concentrations	**H_2O_2**	**FRAP**	**DPPH**	**H_2O_2**	**FRAP**	**DPPH**	**H_2O_2**	**FRAP**	**DPPH**
10 µg/ml	12.53	3.33	59.65	41.50	59.65	50.39	62.39	11.25	69.31
20 µg/ml	15.32	5.83	62.43	46.23	62.43	53.30	64.62	16.67	72.08
30 µg/ml	21.72	9.16	64.28	48.46	64.28	61.50	67.68	31.67	75.66
40 µg/ml	37.04	12.08	65.34	51.81	65.34	66.40	71.58	37.92	78.04
50 µg/ml	39.55	15.41	66.40	56.54	66.40	70.50	76.60	52.08	82.01

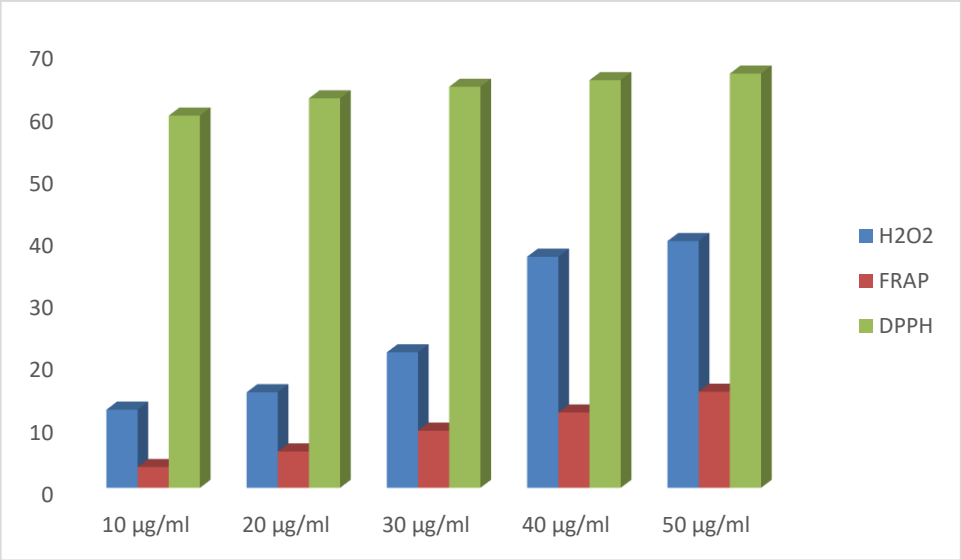

Figure 1. The in vitro antioxidant effect of the crude extracts using H_2O_2, FRAP and DPPH assays

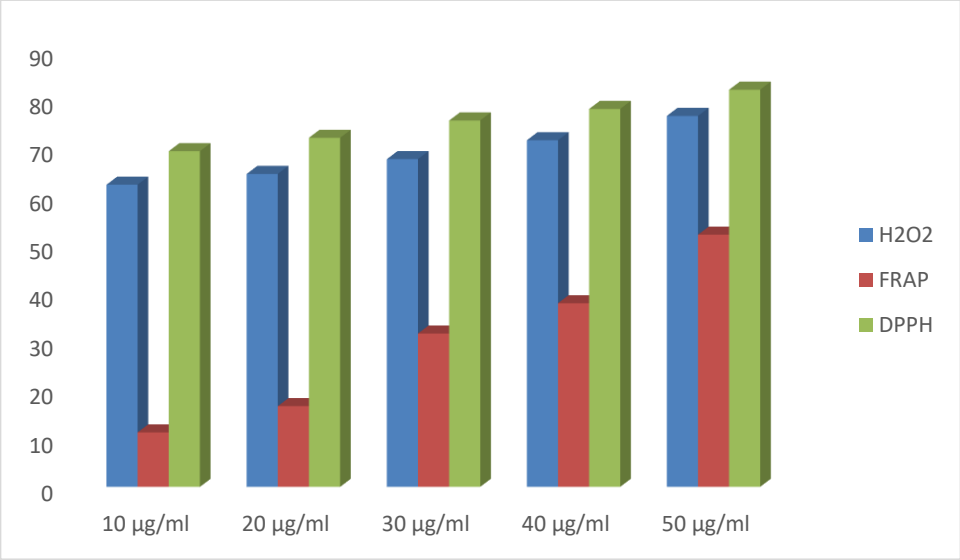

Figure 2. The in vitro antioxidant effect of the essential oil using H_2O_2, FRAP and DPPH assays

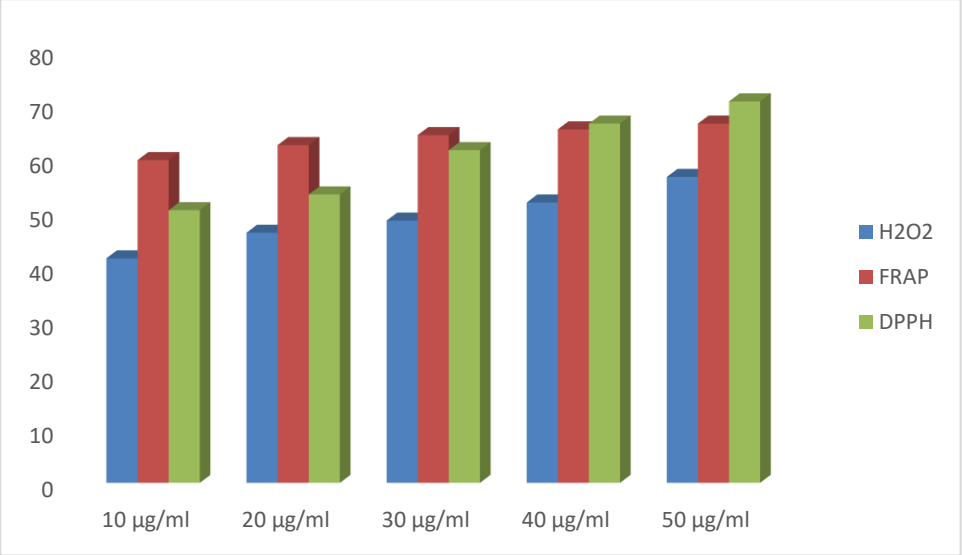

Figure 3. The in vitro antioxidant effect of Ascorbic acid using H_2O_2, FRAP and DPPH assays

The variations in antioxidant activity among different solvents and concentrations of the crude extracts were noteworthy. It was observed that polar solvents, such as methanol, exhibited higher antioxidant activity compared to non-polar solvents like hexane **(**Sonia *et al*., 2013; Judzentiene *et al*., 2023**)**. This suggests that the extraction solvent plays a crucial role in extracting bioactive compounds with antioxidant potential from *Leucas Martinicensis* (Tagnaout *et al*., 2020). The DPPH, H_2O_2 and FRAP results were represented in the graphs below.

Conclusion

The findings of this study contribute to the understanding of the chemical constituents and biological activities of *Leucas Martinicensis*, supporting its potential as a valuable medicinal plant. Further exploration of its phytochemical composition and therapeutic properties may lead to the development of new natural products with significant health benefits and applications in the pharmaceutical and food industries.

Acknowledgement: The technical inputs of Dr. Muhammad Yahaya of AUN, Yola are acknowledged.
Disclosure statement: *Conflict of Interest:* The authors declare that there are no conflicts of interest.
Compliance with Ethical Standards: This article does not contain any studies involving human or animal subjects.

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