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ORIGINAL ARTICLE

Optimization of extraction and evaluation of antiradical and antityrosinase activities of *Diodia sarmentosa* Sw (Rubiaceae) and its major constituent oleanolic acid

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Abstract: *Diodia sarmentosa*, also known as tropical buttonweed, is used traditionally as a culinary ingredient and medicinal plant. This work focused on the determination of optimum conditions for extraction with high yields and evaluation of antityrosinase and antiradical properties of *D. sarmentosa*. Optimization of extraction was performed using response surface methodology (RSM) based on a central composite design. Three variables including mass of plant material, extraction time and ethanol proportion were used for the extraction under ultrasound-assisted conditions and the major compound was isolated and characterized using ^{1}H NMR and ^{13}C NMR as oleanolic acid (OA). The yield of extraction was significantly influenced by mass of plant material, ethanol ratio and extraction time. Highest extraction yield of 40.42% was obtained from 850 g of plant material for an extraction time of 20 mins with ethanol proportion of 80% in water. The extracts resulting from experiments with best yields 40.42% and 37.02% were exhibited good antiradical property with IC_{50} values of 08.1 ± 0.5 µg/mL and 08.7 ± 0.4 µg/mL in the DPPH assay. The extract with a yield of 28.47%, obtained from 850 g of plant material, after 8 mins of extraction time in 80% ethanol proportion had highest tyrosinase inhibition with IC_{50} value of 52.7 ± 1.4 µg/mL. These suggest that mass of plant material and ethanol proportion are important for tyrosinase inhibition and antiradical activities. Oleanolic acid showed very weak antiradical activity with moderate tyrosinase inhibition. Molecular docking showed low binding affinity with the binding sites of tyrosinase, mostly through Van der Waals interactions with a binding energy of −6.3 kcal/mol. However, the overall results indicate potential cosmetic and food applications of *D. sarmentosa*.

Keywords: *Diodia sarmentosa*, optimization of extraction, radical scavenging, tyrosinase inhibition, oleanolic acid

1 Introduction

Food plants, herbs, and spices that contain bioactive substances like polyphenols, terpenes, and alkaloids are known as medicinal plant foods. These substances have been shown to improve immunity, combat infection, and reduce inflammation. Medicinal plants and their activesubstances have therapeutic properties that have been exploited over time by various populations to treat a variety of illnesses (Aye et al., 2019). Hyperpigmentation is an aesthetic skin problem that results into photoaging and melanogenesis, and some medicinal plants with antioxidant and antityrosinase properties are used in cosmetic products to protect the skin and provide whitening effects (Chunhakant & Chaicharoenpong, 2019; Tung et al., 2023; Sytykiewicz et al., 2025). Antiradical and antityrosinase activities refer to the ability of a chemical to both neutralize free radicals and inhibit the enzyme tyrosinase, respectively (Chang et al., 2011; Beddiar et al., 2021; Alain et al., 2022). These qualities are often sought after in cosmetic and food items for their potential benefits in

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avoiding skin aging and browning, respectively (Park et al., 2015; Tamfu et al., 2023).

Antioxidants from plant and plant derived products are gaining interest because of their protective roles in pharmaceutical and food products against oxidative stress and damage in living systems (Tamfu et al., 2022c; Gulcin, 2025). Reactive oxygen species play important physiological roles when in normal amounts but can be excessive due to generation from oxygen metabolism, UV, ionizing radiations, pollutants, heavy metals and xenobiotics causing the imbalance that leads to damage and oxidative stress (Pizzino et al., 2017; Talla et al., 2017; Tumilaar et al., 2024). Free radicals in particular, can be generated through endogenous processes, such as immune cell activation and mitochondrial respiration by exogenous effects including pollution, radiation and smoking and they contribute to the onset of many human diseases (Chandimali et al., 2025). Oxidative stress occurs when there's an imbalance between free radicals (unstable molecules) and antioxidants in the body, leading to cell and tissue damage (Sies, 2020; Zohra et al., 2025). Neutralizing reactive oxygen and nitrogen species (free radicals) can contribute in preventing oxidative stress and damage of biomolecules, thereby reducing the risk of many diseases (Lobo et al., 2010; Feunaing et al., 2024; Haouam et al., 2023).

Skin health is currently gaining attention especially concerning excessive melanin in the skin which results into hyperpigmentation and melanogenesis (Ni et al., 2025). Tyrosinase copper-containing enzyme which plays a key role in enzymatic browning of fruits and vegetables as well as melanin biosynthesis and melanogenesis (Zolghadri et al., 2019; Tamfu et al., 2020b; Tamfu et al., 2025b). As a result, tyrosinase inhibitors are used as depigmenting agents in the pharmaceutical and cosmetics industries as well as antibrowning substances in the food and agricultural sectors (Zolghadri et al., 2019).

Tropical buttomweed, with scientific name *Diodia sarmentosa*, is a scrambling perennial herb without a true root that belongs to the Rubiaceae family (Ekpo et al., 2019; Chinedu et al., 2020). Aerial parts of *D. sarmentosa* including stems and leaves are often used as staple food or for culinary purposes as well as in traditional medicine to treat oedema, dysentery, ulcers, eczema, oedema, injuries, haemorrhoids and pile (Umoh et al., 2016; Soladoye et al., 2010). *Diodia sarmentosa* has exhibited in experimental research to possess antioxidant, anti-inflammatory, antiulcer, analgesic and anti-diabetic properties (Chinedu et al., 2020; Umoh et al., 2016; Elechi et al., 2020). The leaves ethanol extracts of *Diodia sarmentosa* was evaluated on biochemical, antioxidant and histopathological indices of monosodium glutamate-induced uterine leiomyoma in rats (Ezejiofor & Okoroafor, 2022). In another study, *Diodia sarmentosa* leaves exhibited anti-diarrhoea properties in castor oil-induced diarrhoea in albino rats (Anyanwu-Azuka et al., 2021).

As part of our ongoing work on the research of natural enzyme inhibitory substances, this work focused on the optimization of extraction of *D. sarmentosa* and evaluation of antiradical and antityrosinase activities. The major compound of *D. sarmentosa* was also isolated and identified as oleanolic acid.

2 Materials and Methods

2.1 Plant Material and Extraction

Aerial parts of tropical buttomweed, *Diodia sarmentosa* were harvested from the campus of University of Yaounde II Soa, Yaounde in Cameroon during the month of May 2020. The plant was identified under the herbarium number 5123/HNC at the national herbarium of Cameroon. The plants were dried in the shade for three weeks and ground into powder and subjected to extraction.

The extraction was performed using the ultrasonic extraction as described elsewhere with some modifications (Tamfu et al., 2022b; Tamfu et al., 2022a). The powdered plant material in variable amounts (60.6–1039.9 g) were mixed with water (H_2O): ethanol (EtOH) in variable proportions (0.101–9.899 EtOH). The mixture was subjected to ultrasonic extraction using a Caliskan ultrasonic cleaner bath (LAB.ULT.4030) at 40 kHz for variable preset extraction time (4.202–23.798 mins) according to the experimental design. Each experiment was done in triplicate and the supernatant was collected, filtered and evaporated at 50 °C using a Rotavapor to afford variable amounts of crude extract.

2.2 Experimental Design for Extraction

In our work, the response surface methodology was used and allowed in evaluating the effects of three independent variables (mass X_1, time X_2 and ethanol proportion X_3) in the yield of extraction. The center composite design was used to fix the levels of variation of the different factors (Table 1).

Table 2 shows the experimental design with the responses obtained. For each experiment, the result (percentage yield of extraction) represents the average of three experiments. With three factors, the mathematical model used is that of the second degree with interaction between the different parameters and is presented in the form:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X^2_1 + b_{22}X^2_2 + b_{33}X^2_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

Minitab 15 statistical software was used to determine the coefficients of the model by the least squares method (Myers & Montgomery, 2002) and also to perform the statistical analyses of variance (ANOVA).

2.3 Isolation of Major Compound

In most of the extraction experiments, crude precipitates were formed at the bottom of the vials upon standing. They were filtered and subjected to thin layer chromatography (TLC) using pre-coated silica gel (60 F254) on aluminium support (Merck) and revealed under UV lamp at 254 nM and 365 nM followed by spraying with diluted sulfuric acid and heating. From the TLC profiles, a major component was observed in almost all of the precipitates. 2 g of this crude precipitate was subjected to column chromatography

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Table 1. Values of independent variables with their levels of variation

Variable	Symbol	Level of variation				
		−1.633	**−1**	**0**	**+1**	**+1.633**
Mass (g)	X_1	60.1	250	550	850	1039.9
Time (min)	X_2	4.2	8	14	20	23.7
Solvent ratio (EtOH proportion in 10 mL)	X_3	0.1	2	5	8	9.8

Table 2. Experimental design and obtained responses

Experiment	Variables			Response
	Mass (X_1)	**Time (X_2)**	**Solvent ratio (X_3)**	**% Extraction**
1	550	14	0.101	08.91
2	550	4.202	5	09.54
3	550	14	5	20.36
4	550	14	9.899	22.73
5	1039.9	14	5	37.02
6	550	14	5	20.36
7	550	23.798	5	23.45
8	60.1	14	5	18.33
9	550	14	5	20.36
10	850	8	2	21.18
11	850	20	2	23.53
12	250	20	8	16.80
13	850	8	8	28.47
14	250	8	8	23.20
15	550	14	5	20.36
16	550	14	5	20.36
17	550	14	5	20.36
18	250	8	2	17.20
19	250	20	2	20.80
20	850	20	8	40.24

using silica gel 70–230 mesh (Merck, Darmstadt, Germany), as stationary phase and an isocratic eluent composed of n-hexane:ethyl acetate (8:2) to afford 32 fractions in which oleanolic acid (87 g) were filtered out in pure form.

2.4 DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the samples was determined by the DPPH assay described elsewhere, with slight modifications (Tamfu et al., 2020a). In its radical form DPPH absorbs at 517 nM, but on reduction by an antioxidant or a radical species its absorption decreases. Briefly, a 0.1 mmol L^{-1} solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of samples solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nM. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_{\text{Control}} - A_{\text{Sample}}}{A_{\text{Control}}} \times 100$$

where A_{Control} is the initial concentration of the DPPH and A_{Sample} is the absorbance of the remaining concentration of DPPH in the presence of the extract and positive control. BHT and a-tocopherol were used as antioxidant standards, for comparison of the activity. The sample concentration providing 50% free radical scavenging activity (IC_{50}) was calculated from the graph of DPPH Scavenging effect percentage against sample concentration.

2.5 Anti-Tyrosinase Activity

Tyrosinase enzyme inhibitory activity was measured by the spectrophotometric method as described previously (Masuda et al., 2005). Mushroom tyrosinase was used, while L-DOPA was employed as substrates of the reaction. Briefly, 150 µL of 100 mM sodium phosphate buffer (pH 6.8), 10 µL of sample solution dissolved in ethanol at different concentrations, and 20 µL tyrosinase enzyme solution in buffer were mixed and incubated for 10 min at 37°C, and 20 µL L-DOPA was added. The absorbance of sample and blank were read at 475 nM after 10 min incubation at 37°C in a 96-well microplate. The results were given as inhibition

percentage (%) of the enzyme at 200 µg/mL concentration of the extracts.

2.6 In Silico Evaluation

Agaricus bisporus tyrosinase structure (PPO3) was used as the receptor, starting from the 2Y9X crystallographic model and retaining chain A, which houses the catalytic domain and the dicopper active site (Ismaya et al., 2011). The crystallographic inhibitor (tropolone) was removed prior to docking, while both copper ions were retained to preserve metal-site geometry and electrostatics. To avoid preparation artefacts around the metal, the bridging solvent was omitted for AutoDock Vina docking, with the understanding that it can be reinstated for subsequent molecular dynamics. The docking grid was centered at the midpoint of the two copper coordinates, first sampled with a 24 × 24 × 24 Å cube for exploratory runs and then narrowed to 20 × 20 × 20 Å to reduce off-site placements. The center of the molecular docking site is defined at −10.032; −28.769 and −43.467 as X, Y, Z dimensions respectively (Desmiaty et al., 2021). Ligands were prepared from SMILES for both neutral OA (–COOH) and the anion ($–COO^-$); tropolone served as a positive control. SwissDock was used with AutoDock Vina (Trott & Olson, 2010) as the primary engine at increased exhaustiveness (12), and Attracting Cavities 2.0 (Rohrig et al., 2023) was run as a cross-check (SwissDock 2024 implementation: Bugnon et al., (2024). Poses were classified as non-productive if all OA oxygen–copper distances were > 3.3 Å or if they showed unrealistic bridging across both metals. Poses with a single O-Cu approach in the 2.2–3.0 Å range would have been flagged for MD follow-up; none of the OA poses met that criterion. Separately, OA was profiled with SwissADME (Daina et al., 2017), pkCSM (Pires et al., 2015), and DeepPK (Myung et al., 2024) for physicochemical constraints, permeability, clearance, and toxicity flags relevant to topical or cellular testing.

2.7 Statistical Analysis

Activity assays were performed in triplicate analyses. The data were recorded as means ±Standard Error of the Means (SEM). Student's *t*-test performed using Minitab 15 statistical software were used to determine the significant differences between means, that is $p < 0.05$ were regarded as significant.

3 Results and Discussion

3.1 Extraction Parameters

Ultrasound-assisted extraction is used as an efficient tool for the green extraction of bioactive excipients from plants. In addition to using ultrasonication, extraction time, solvent composition and amount of plant material are important parameters that can be optimized for efficient and high yield of extraction. The response surface methodology (RSM) is used as a suitable tool for the planning and extraction under optimal conditions. This RSM was used as a mathematical tool to evaluate and understand the interactions of the different parameters or their collective impact on the extraction yield of *D. sarmentosa*. This approach reduces wastage of extraction solvents and saves time in addition to the optimization of extraction of active metabolites from plants. The experimental design, the parameters as well as their variations and the percentage of extraction which is the response are summarized on Table 2 below.

The experimental data was analyzed and best fitted model was selected for optimization studies. The statistical model was selected on the bases of R^2 coefficient, lack of fit test and sequential model p-value. The analysis of variance and regression coefficients of the second degree polynomial model on the percentage of extraction yield are presented on Table 3. P-values less than 0.0500 indicate model terms are significant. In this case X_1 X_2 , X_3, X_1 X_1 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Many factors including temperature, solvent types, solid-liquid ratio, extraction pressure and time can affect the extraction efficiency significantly (Sai-Ut et al., 2023). The process of optimizing extraction *D. sarmentosa* involved modifying extraction parameters including mass of plant material, time of extraction and solvent ration with the aim of maximizing the yield of extraction which was the response. Polarity of solvent such as EtOH proportion, extraction duration, mass of plant material and temperature are most often the key parameters (Pascariu et al., 2024; Toy et al., 2025).

3.2 Contribution of the Different Variables on the Extraction Percentage and RSM Plots

The coefficients on Table 3 are inserted into the equation of the model:

$$Y = 20.04 + 3.90X_1 + 3.60X_2 + 4.39X_3 + 3.95X^2{}_1 - 0.2433X^2{}_2 - 0.4964X^2{}_3 + 0.8650X_1X_2 + X_1X_3 + 1.68X_2X_3$$

The analysis of the model equation shows that the linear effects of mass, time and extraction solvent ratio (X_1, X_2 and X_3) have positive influences. On the other hand, the quadratic effects ($\mathbf{X^2_2}$ **et** $\mathbf{X^2_3}$) have negative coefficients, indicating that the extraction percentage decreases when these parameters increase. A positive influence of extraction time and extraction solvent ratio (X_2 and X_3) and the negative influence of their square terms (X_2X_2 and X_2X_3) was observed. This may be due to the fact that when the extraction time is long, the extraction medium become saturated and extraction stops or slows. Also, when the solvent ratio is high, the extracted compounds may have less affinity for ethanol, thus decreasing the extraction yield. However, all the parameters have a positive influence on the extraction yield. This is explained by the fact that the extraction yield of compounds is closely linked to the mass of the plant material, the extraction time and the nature of the extraction solvent.

This study reports the effect of three independent variables (mass of plant material, time of extraction and solvent ratio). The results suggest that the mass of plant material and the ration of ethanol in water greatly influenced the extraction yield. However, extraction yields were also high when longer extraction times were employed. A similar trend was observed when the same variables including water–ethanol

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Table 3. Analysis of variance and regression coefficients of the second degree polynomial model on the percentage of extraction yield

Effect	Df	Coefficient	Sums of squares	F-value	P-value
Linear					
X_1	1	3.90	202.34	16.12	0.0025
X_2	1	3.60	173.05	13.79	0.0040
X_3	1	4.39	257.27	20.50	0.0011
Quadratic					
X_1X_1	1	3.95	205.97	16.41	0.0023
X_2X_2	1	−0.2433	0.7819	0.0623	0.8080
X_3X_3	1	−0.4964	3.25	0.2593	0.6216
Interaction					
X_1X_2	1	0.8650	5.99	0.4769	0.5055
X_1X_3	1	1.00	8.00	0.6374	0.4432
X_2X_3	1	1.68	22.51	1.79	0.2101
X_0	1	20.04			
Pure error	5	0.0000	0.0000		
Lack of Fit	5	125.51	25.10		
Total	20	1011.66			
R^2		0.0506			
R^2 - adjusted		0.7643			

Note: X_1 *mass;* X_2 *extraction time;* X_3 *solvent ratio;* X_1X_1 *mass x mass;* X_2X_2 *time x time;* X_3X_3 *solvent ration x solvent ration;* X_1X_2 *mass x extraction time;* X_1X_3 *mass x solvent ration;* X_2X_3 *extraction time x solvent ration. Xo constant.*

percentage, fruit amount and time were evaluated for their optimized effects for maximizing quercetin and gallic acid contents in *Pouteria macrophylla* fruits (Albuquerque et al., 2025). Optimization of extraction is advantageous in that it can minimize expenses and time, and lessen the impact on the environment. In addition, the process of optimizing extraction increases extraction yield with response surface methodology being the most employed statistical tool for efficient multifactorial optimization (Vern Ng et al., 2025). Optimization using response surface methodology is often used in extracting bioactive compounds from plants especially for antioxidant applications (Robles-Apodaca et al., 2024; Taibi et al., 2025).

The optimization process is presented on the 3D response surface plots of significant first order interaction terms for the response which is the percentage extraction yield as shown on Figure 1. The combined effect of extraction time and the mass of plant material suggests that the maximum percentage of extraction is obtained when maximum extraction time and highest amount of plant material was provided. The 3D plot indicates that increasing the amount of plant material and the extraction time increases extraction yield. The 3D plot between solvent ratio (ratio of ethanol in 10 mL of solvent mixture) and mass of plant material shows that median solvent ratio and highest plant material mass provides optimum extraction yield.

3.3 Characterization of the Major Constituent, Oleanolic Acid

The major constituent of *D. sarmentosa* was extracted and characterized using ^{1}H NMR and ^{13}C NMR as oleanolic acid whose structure is provided in Figure 2.

^{1}H NMR ($CDCl_3$, 400 MHz): δ_H ppm; H-1 (1.57), H-2 (1.93), H-3 (3.20, dd), H-5 (0.88, d), H-6 (1.54, m), H-7 (1.37, m), H-9 (1.67, m), H-11 (1.96, dd), H-12 (5.23, t, J = 3.7 Hz), H-15 (2.18), H-16 (1.94, dd), H-18 (2.73, dd, J = 13.9; 4.4 Hz), H-19 (1.4), H-21 (1.44), H-22 (2.06, m), H-23 (1.33, s), H-24 (0.97, s), H-25 (0.77, s), H-26 (0.96, s), H-27 (1.11, s), H-29 (0.84, s), H-30 (0.95, s). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C C-1 (39.0), C-2 (28.1), C-3 (78.9), C-4 (38.4), C-5 (54.8), C-6 (18.0), C-7 (32.4), C-8 (38.5), C-9 (47.2), C-10 (36.6), C-11 (22.8), C-12 (121.5), C-13 (143.7), C-14 (40.9), C-15 (28.4), C-16 (25.6), C-17 (45.7), C-18 (41.4), C-19 (45.6), C-20 (30.5), C-21 (33.3), C-22 (32.5), C-23 (28.2), C-24 (16.0), C-25 (15.1), C-26 (16.9), C-27 (26.9), C-28 (178.9), C-29 (32.8), C-30 (23.8).

3.4 Radical Scavenging Activity

The extracts obtained from the various extraction procedures were evaluated for their radical scavenging effects against DPPH and the results expressed in terms of IC_{50} values and plotted on Figure 3. The highest radical scavenging activity was exhibited by the extracts from experiment 5 (IC_{50} = 08.1 ± 0.5 µg/mL) and experiment 20 (IC_{50} = 08.7 ± 0.4 µg/mL). In experiment 5, the amount of plant material was 1039.9 g, with extraction time of 14 mins in a solvent ratio which had 50% ethanol in water and yielded 37.02% of extract. In experiment 20, the mass of plant material was 850

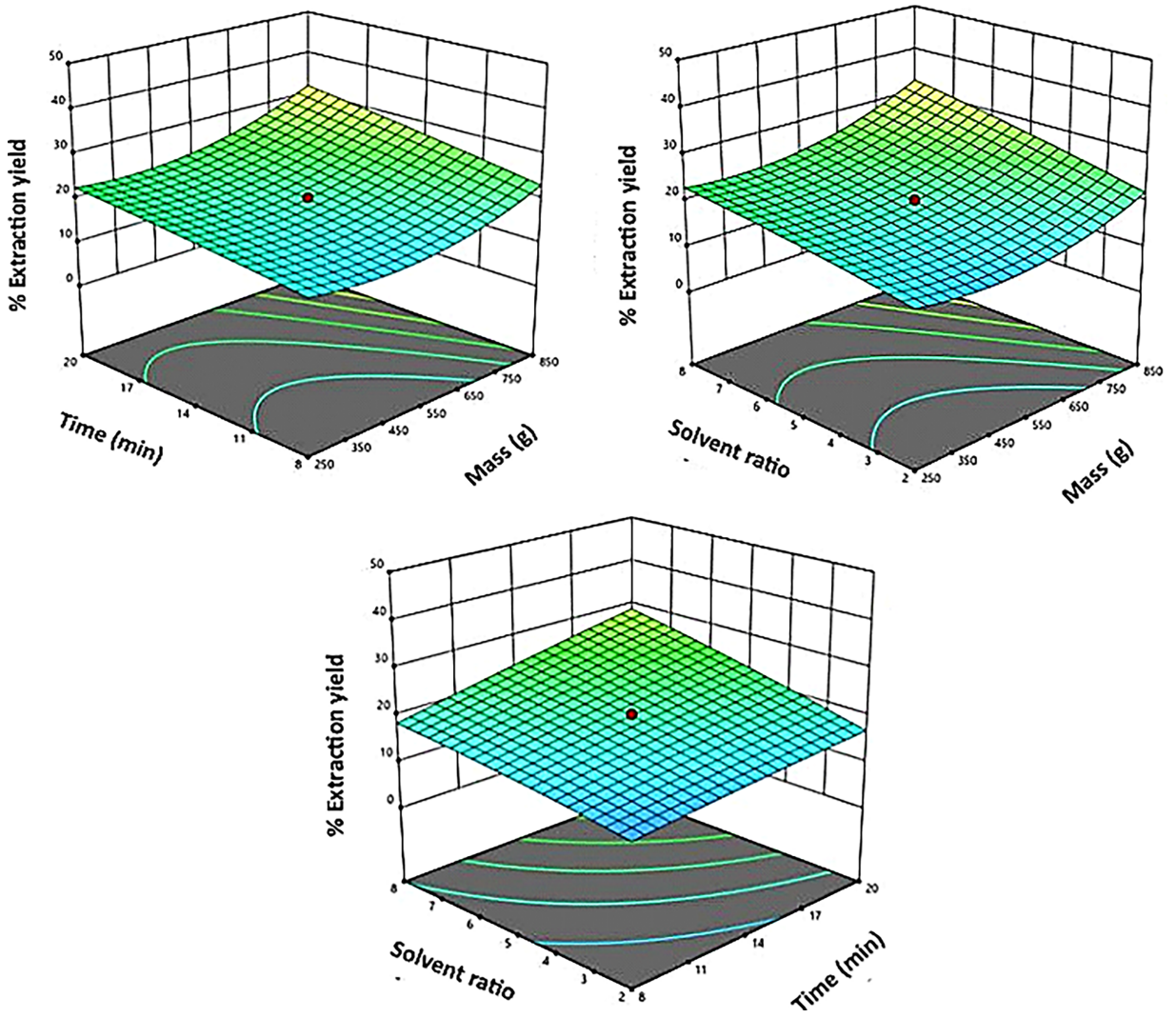

Figure 1. 3D response surface methodology plots of significant 1st order interaction terms for percentage extraction yield

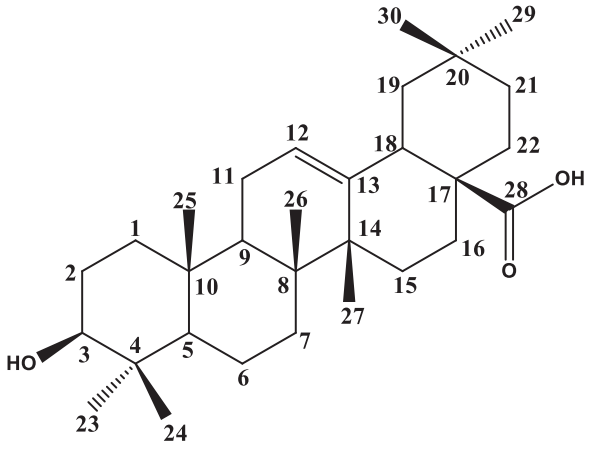

Figure 2. Structure of Oleanolic acid

g with extraction time of 20 minutes and solvent of extraction 80% of ethanol in water with a percentage yield of extraction of 40.24%. The results suggest that, high amounts of plant material and longer extraction times improved extraction yield and radical scavenging activities. Experiment 8 (IC_{50} = 21.6 ± 0.4 µg/mL) had the lowest radical scavenging activity and was obtained from 60.1 g plant material in 14 mins extraction time. Oleanolic acid (OA) had very low activity with IC_{50} = 592.8 ± 2.1 µg/mL.

Parameters including solid-to-solvent ratio, ultrasound extraction time, and ethanol concentration can be optimized to give extracts with good antioxidant activity and high phenolic contents (Sai-Ut et al., 2024). The extracts of *D. sarnentosa* obtained from the optimization of extractive parameters which were mass of plant material, ultrasound extraction time and ethanol concentration exhibited antioxidant activity. Extracts resulting from experiments 5 and experiment 20 had the best antiradical activity against DPPH. These experiments 5 and 20 used high amounts of plant material of 1039.9 g and 850 g respectively, as well as longer ultrasound extraction times of 14 mins and 20 mins respectively. This suggests that the mass of plant material and the extraction times play important role in the extraction of antioxidant compounds from *D. sarmentosa*. Liquid-solid

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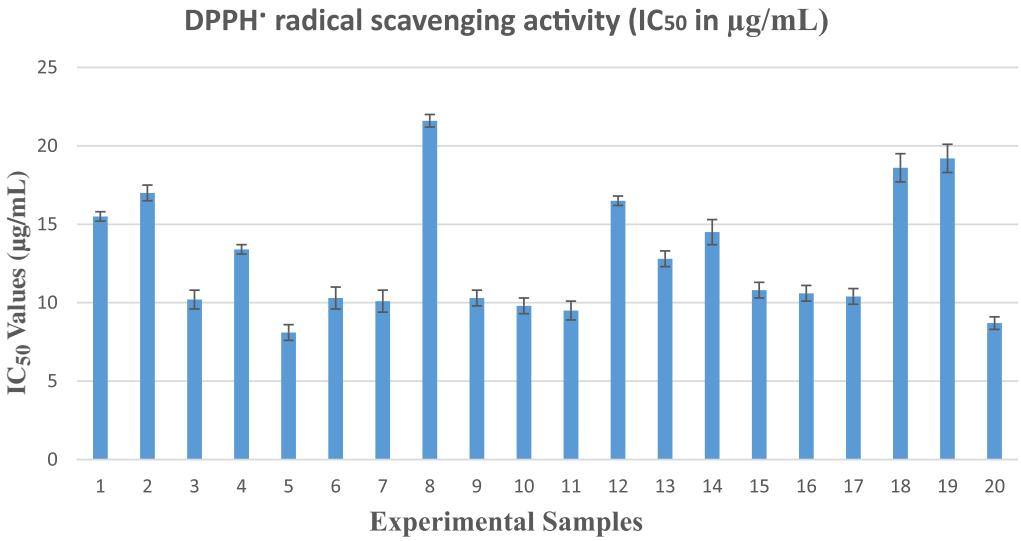

Figure 3. DPPH radical scavenging activity

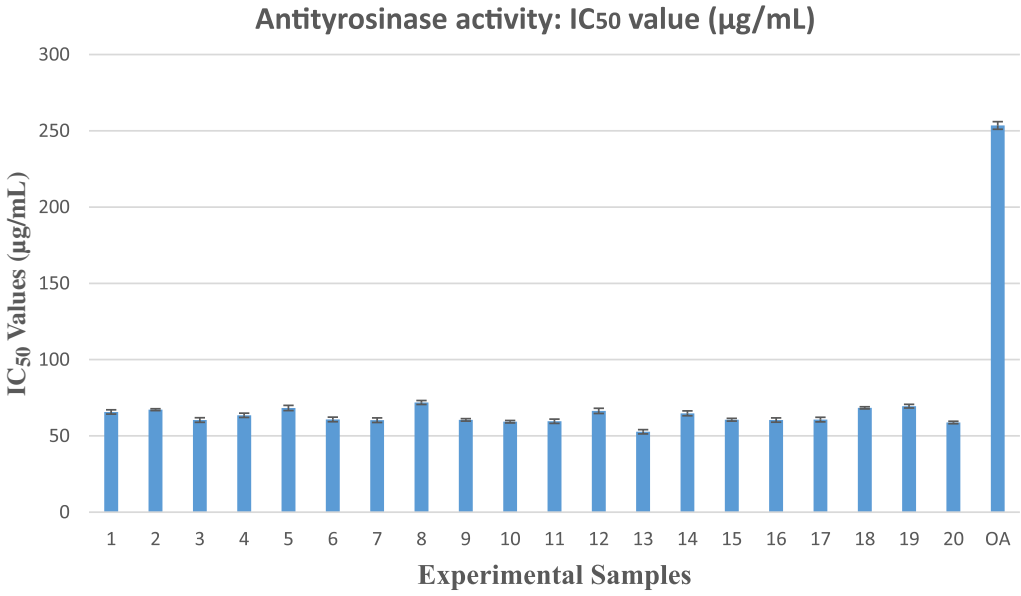

Figure 4. Tyrosinase inhibitory activity

ratio, solvent ratio and extraction time have been shown to be the most important parameters that affect the extraction of antioxidant compounds (Majeed et al., 2016; Yang & Li, 2022). Variables such as extraction time and solvent mixture are suitable for extraction of antioxidant compounds especially when they are used together with ultrasound-assisted appliance (Michalaki et al., 2023). Preparation of antioxidant extracts is very important as they find applications in food, cosmetic and pharmaceutical industries (Kotha et al., 2022; Sawalda et al., 2022). Antioxidant subtsances are capable of neutralizing unstable radical species in living systems and protecting biomolecules and cells from oxidative stress damage, preventing diseases such as cancers, diabetes, cardiovascular diseases and others (Chandimali et al., 2025; Robles-Apodaca et al., 2024; Taibi et al., 2025; Sai-Ut et al., 2024; Majeed et al., 2016; Yang & Li, 2022; Michalaki et al., 2023; Kotha et al., 2022; Sawalda et al., 2022; Zehiroglu & Ozturk Sarikaya, 2019; Boudiba et al., 2023; Gulcin, 2025). The antiradical activity of the extracts of *D. sarmentosa* indicate their beneficial property to human health as they can be used to defend the body from the destructive effects of free radicals. The antiradical potential demonstrated by the extracts obtained in the study is in agreement with previous report which showed, indicating that aqueous and ethanol extracts of *D. sarmentosa* significantly inhibit 2,2-diphenyl-1-picrylhydrazyl radical with IC_{50} values of 10.121 and 10.994 µg/mL (Chinedu et al., 2020). This is relatively similar to the IC_{50} values obtained in this study which ranged from 08.1 µg/mL to 21.6 µg/mL.

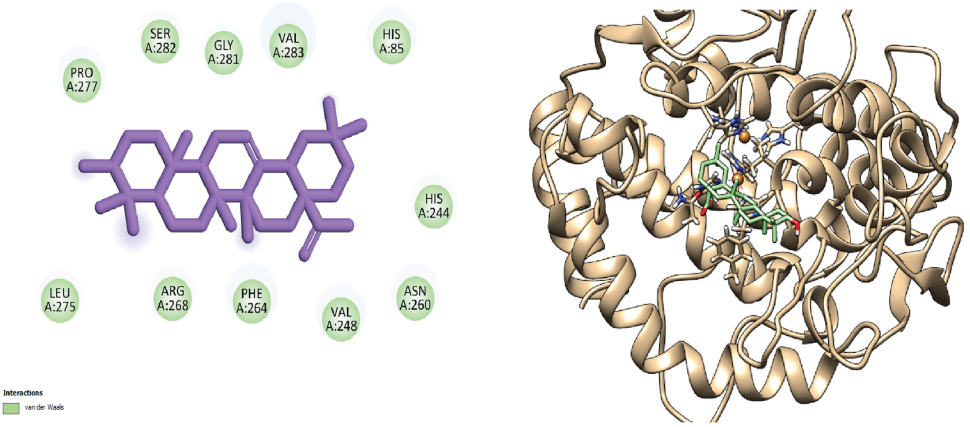

Figure 5. 2D and 3D view of OA interacting with tyrosinase enzyme (pdb id: 2Y9X)

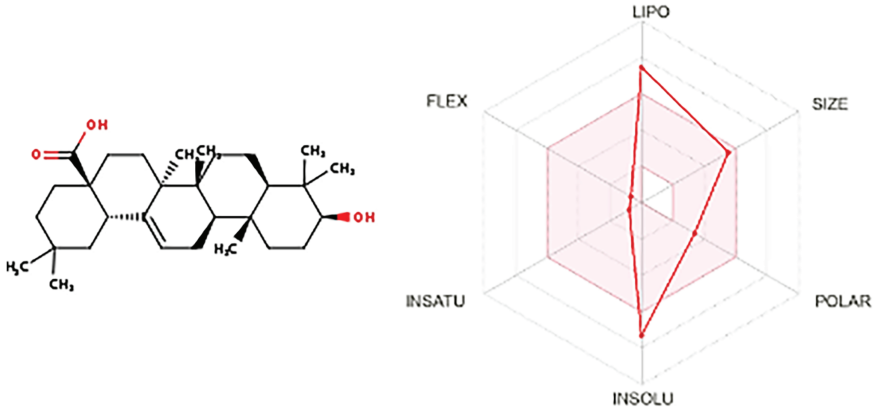

Figure 6. Oral bioavailability radar map of OA

3.5 Tyrosinase Inhibitory Activity

The different extracts were evaluated for their ability to inhibit the copper-containing metallo-enzyme tyrosinase and the results presented in terms of IC_{50} values on Figure 4. The extracts were more active than the major compound oleanolic acid (OA) which had a low IC_{50} value of 253.9 ± 2.5 µg/mL. The most active extract was that obtained from experiment 13 which exhibited IC_{50} value of 52.7 ± 1.4 µg/mL. This was obtained from a high amount of plant extract (850 g) and an average extraction time (8 mins) with a high ethanol proportion (80%) in water, giving an extraction yield of 26.47%. This suggests that high plant material and ethanol percentage could improve tyrosinase inhibition. This tendency was exhibited by the extract from experiment 20 which was obtained from 850 g of plant material in 80% ethanol in water, having and IC_{50} value of 58.8 ± 0.8 µg/mL.

Antioxidant activity and enzymatic activity are interrelated and most substances which possess antiradical potential also show enzymes inhibitory activity (Koudoro et al., 2023). Radical species can be generated by ionizing radiation, affecting the skin and making antioxidants to find applications in cosmetic products. Inside the cells, the radicals can secondary oxidation and redox reactions with some metal ions such as iron and copper (Tumilaar et al., 2024). The tyrosinase is a copper-containing metallo-enzyme, which produces melanin and causes enzymatic browning in a variety of organisms (Munvera et al., 2023; Benaissa et al., 2025a). The extracts obtained from the designed experiments equally showed interesting antityrosinase activity. The extract with the highest tyrosinase inhibition resulted from experiment 13 in which the mass of plant extract was high (850 g), with average extraction time (8 mins) and high EtOH proportions (80%). It is noteworthy to mention that the experiment had a relatively high extraction yield (26.47%). The extracts showed good to moderate antioxidant and antityrosinase properties. The major compound, oleanolic acid (OA) had very low antiradical property but exhibited moderate tyrosinase inhibition. Tyrosinase inhibitors find applications as skin-whitening agent in cosmetics and also food and agriculture as preservatives to avoid browning of fruits and vegetables (Ni et al., 2025; Alfred Ngenge et al., 2021). Many antioxidants also have a synergistic effect with enzyme inhibitors, which means that when combined, they increase tyrosinase inhibition, lowering melanin generation and avoiding food discoloration and skin hyperpigmentation (Wang et al., 2016; Cui et al., 2018). Antioxidants are useful in cosmetic and agricultural applications because of this link.

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3.6 In Silico Predictions

The 2D and 3D interactions of major compound of *D. sarmentosa*, OA with the receptor sites of tyrosinase are presented in Figure 5 while the bioavailability radar is presented in Figure 6. Re-docking of tropolone reproduced favorable binding energies (−6.0 kcal/mol) and the expected placement adjacent to the di-copper site, supporting the receptor preparation and grid definition, RMSD to the crystallographic pose was 1.2 Å. In contrast, OA's best score (−6.3 kcal/mol) corresponded to a pose showing only Van der Waals contacts in the interaction viewer, and direct measurements indicated no O-Cu distances in coordination range. Modeling the carboxylate anion did not improve metal proximity, and attracting cavities sampling converged to hydrophobic placements rather than a metal-engaged pose. SwissADME reported a consensus cLogP value of 6 and TPSA 58 $Å^2$ with very poor aqueous solubility and minimal flexibility, while pkCSM and Deep-PK agreed on likely CYP3A4 substrate behavior and flagged potential hepatotoxicity/transport interactions in some models.

Molecular docking and drug-likeness computed through computational means supplements in vitro assay and gives insights into structure-activity relationship (Metiefeng et al., 2023; Benaissa et al., 2025b; Tamfu et al., 2025a). The major compound from *D. sarmentosa*, OA, showed binding affinity most through weak Van der Waals interactions with a binding score of −6.3 kcal/mol. OA showed no interaction with the metal atom of tyrosinase. Together these observations suggest that OA tends to occupy hydrophobic subpockets or entry-channel surfaces without productively engaging the dicopper core (Ismaya et al., 2011). The key point is that OA fails a geometry-based definition of active-site binding even when it's docking score looks reasonable. For metallo-enzymes like tyrosinase, score alone is insufficient; a mechanistically credible pose requires correct orientation and donor-atom proximity to the metal center (validated here by tropolone's re-dock). The bioavailability radar indicates that lipophilicity and insolubility fall out of the pink zone of the polygon. OA's physicochemical profile includes high lipophilicity, low solubility, and limited polarity favors membrane partitioning and hydrophobic surface occupancy over specific dicopper coordination (Daina et al., 2017; Pires et al., 2015; Myung et al., 2024). Practically, if cellular assays show melanogenesis modulation by OA, mechanisms such as signaling, redox buffering, or membrane effects are more plausible than direct copper chelation.

4 Conclusion

The importance of medicinal plants in pharmacological, cosmetic, food and agricultural sectors continue to attract attention and necessitating proper extractive methods, chemical analyses and bioassays. *D. sarmentosa* is one example of such plants. Preparation of extracts from *D. sarmentosa* was successfully optimized response surface methodology and indicated that, mass of plant material, extraction time and ethanol proportion were significant parameters influencing the extraction process. Oleanolic acid was isolated and characterized as the major compound from the *D. sarmentosa* extracts. The extracts exhibited antiradical activity with the extracts resulting from experiments with high mass of plant material and longer extraction times had highest extraction yields while showing highest activity. The extracts equally showed antityrosinase effect and the results suggest that high amounts of plant material and ethanol ration could influence the activity. Though oleanolic acid showed low tyrosinase inhibitory activity, it exhibited a negative binding energy, indicating that it could bind spontaneously to tyrosinase receptor sites, possibly through Van der Waals interactions. The antiradical and antityrisonase properties of the extracts suggest that they could be used as whitening agents in cosmetics or preservatives for fruits and vegetables.

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Availability of Data and Materials

The authors declare that should any raw data files be needed about the further data of the study, they are available from the corresponding author upon reasonable request. Source data are provided with this paper.

Conflict of Interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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