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Original Research Article

In-Vitro Assessment of Skin Enzymes Inhibitory Activities, Total Antioxidant Potentials and Ultraviolet Spectral Characteristics of *Chromolaena odorata* Methanol Extract and its Column Chromatographic Fractions

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ABSRTACT

In Nigeria, many locally useful plants go into extinction unnoticed. Notably, *Chromolaena odorata* has been employed in ethnomedicine for several therapeutic benefits, particularly for treating external wounds, skin infections, and inflammation, among others. The in-vitro evaluation of the UV spectrum properties, total antioxidant potentials, and inhibitory activities of skin enzymes of *Chromolaena odorata* methanol extract and its column chromatographic fractions are presented in this study. Methanolic (70%) extracts of *Chromolaena odorata* were subjected to TLC profiling based on the separation pattern, that is, inferred with the determination based on the ratio of solvent systems consisting of hexane: ethylacetate (7:3) and dichloromethane: methanol (95:5), and determination using various detection systems. Then, antioxidant activity was tested in an *in-vitro* system using: trolox equivalent absorbance capacity (TEAC), oxygen radicals' absorbance capacity (ORAC), ferric-ion reducing antioxidant power (FRAP), and lipid peroxidation (LPO); while skin degenerative enzymes actions were evaluated using: tyrosinase (TYR); and elastase (ELA). TLC profiling revealed varying degrees of flavonoids and other polyphenolic constituents in the plant. The findings suggest that a range of phytochemicals, which may be employed as natural antioxidants, are abundant in the extract with coefficient correlation difference at $p < 0.05$ in total antioxidant capacities. Furthermore, *Chromolaena* odorata potential as a UV-absorbing agent was demonstrated by its ultraviolet spectral properties. These findings open up new possibilities for the incorporation of *Chromolaena odorata* into skincare products by highlighting the plant's excellent potential as a source of bioactive chemicals with dermatological and cosmeceutical relevance.

Keywords: *Chromolaena odorata*, oxidative stress, chromatography, cosmeceutical, free radical scavenging, skin enzymes.

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Introduction

Discovering and developing novel therapeutic agents has long been inspired by the availability of natural products, especially when it comes to skincare and dermatological health. Herbal-based cosmeceuticals have emerged as a promising avenue for skin protection and regeneration, supported by the traditional use of medicinal plants for skincare applications.[1] Cosmeceutical research has focused heavily on the inhibitory potential of plant-based compounds on skin enzymes such as elastase, and tyrosinase, which implicate hyperpigmentation, skin aging, and wrinkling.[2] Antioxidant properties that showcase the protective potential of these compounds including flavonoids and polyphenols, against oxidative stress-induced skin damage, remain essential.[3-4] Recently, UV-absorbing properties of plant-derived compounds have attracted immense attention to establish their photoprotective potential as alternatives to synthetic sunscreens.[5]

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Numerous tropical regions are home to *Chromolaena odorata* (L.), a weed with a long history of traditional use that has gained attention recently for its wide range of medical benefits.[6] There are reports on several promising pharmacological activities such as antimalarial activity, antimicrobial activity, antibiofilm activity, antihepatotoxicity, anthelmintic activity, antiviral activity phytopathogenic activity, antiprotozoal activity, antioxidant activity, as well as wound healing, associated with *Chromolaena odorata* the methanol extract, suggesting their dermatological applications.[2,4,6-9] There is scientific evidence to support the traditional usage of *Chromolaena odorata* in skincare products since these bioactive components can demonstrate skin-protective properties through their antioxidant potential and inhibition of skin-related enzymes.[9] Furthermore, the extraction and fractionation of bioactive components of *Chromolaena odorata* hold promise in identifying chemical constituents for cosmetic products.[9-11] However, there is a dearth of scientific research examining its UV spectrum properties and inhibitory actions on skin enzymes.

Therefore, by assessing the skin enzyme inhibitory activities, total antioxidant potentials, column chromatographic fractions, and UV spectrum properties, this study sought to close the existing knowledge gap on *Chromolaena odorata* methanolic extract. Consequently, this research will unravel a scientific basis underpinning the traditional use of *Chromolaena odorata* in skin care and wound healing, thereby giving insight into possible bioactive substances for the development of new and more effective natural cosmetic and dermatological formulations.

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Materials and Methods

Acquisition and Preparation of Chromolaena odorata methanol extract
Aerial parts of *Chromolaena odorata* were freshly obtained in July 2022 from Ikere Ekiti [7° 29' 50.93" N; 5° 13' 49.48" E], a southwestern state in Nigeria. The validation of the specimen was carried out at the herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria, and a copy was deposited with herbarium number (UHAE2024004).
Before milling, the plants were allowed to dry for three weeks at ambient temperature in the laboratory. In an aspirator container, the finely powdered plant (1 kg) was macerated for 72 hours with 70 % methanol (7.5 L) in a shaker. The mixture was filtered through a Whatman filter paper (grade 1) and concentrated in a rotary evaporator (R-110, Buchi, Switzerland) under a reduced pressure at 35°C.[12] The resultant brownish-green extract was labeled as FR and stored for subsequent analysis.

Extract Fractionation using Thin Layer Chromatography
Fractionation was done with column chromatographic techniques using silica gel (as the stationary phase) with a gradient elution system that employs organic solvents of hexane (100%); and hexane:ethylacetate (9:1; 7:3; 1:1; 3:7).[13] In a Petri plate, 20 g of concentrated extract of *Chromolaena odorata* was applied onto a silica gel column (23x12 cm) overnight. Fractions from the open column were concentrated and developed on TLC (Merck, Damstadt, Germany) with solvent systems [A (ethylacetate: hexane 3:7) and B (methanol: dichloromethane 5:95)]. The plates were created, and then the vanillin-sulphuric acid spray reagent was used to identify the class of organic compounds in the extract[14]. All TLC chromatograms were initially examined using a CAMAG UV lamp (254nm and 366nm). When sprayed with vanillin/sulphuric acid, fractions with the same TLC properties were merged to generate six (6) major fractions labeled FR (I-VI). The mixed fractions were redeveloped with the two distinct solvent systems, observed under a UV light (254 and 366 nm), and finally sprayed with a freshly made vanillin-sulphuric acid spray solution.[13, 14]

UV Spectral Characteristics and Photoprotection Activity
The maximum absorption wavelength (λ_{max}) of *Chromolaena odorata* methanolic extract was evaluated using the method of Violante et al.[15] The methanol extract (FR) and its column chromatographic fractions (I-VI) were dissolved in absolute ethanol at 1 mg/mL concentrations in duplicate. The UV properties of each sample (1 mg/mL) in 5 mL ethanol were initially measured using a UV Spectrophotometer (JENWAY 7315, United Kingdom) between 200 and 700 nm. After that, a pinch of sodium acetate solid was added to the first set of each sample in a cuvette (5 ml, m/v) and well shaken, while boric acid solid was added to the second set of each sample. The UV spectrum was measured between 200 and 700 nm, and the results were compared to the original measurement without sodium acetate (as in the first set) and boric acid (as in the second set). If a hydroxyl group is present at the C-7 in the presence of sodium acetate, a bathochromic shift in UV max compared to the original spectrum will be observed; if an ortho dihydroxy system is present when boric acid is added, a bathochromic shift in UV max compared to the original spectrum will be observed.[16]

Determination of Ferric-ion Reducing Antioxidant Power (FRAP)
Based on the method of Benzie and Strain,[17] a working FRAP reagent containing tripyridyl triazine (TPTZ) (10 mM in 40 mM HCl), $FeCl_3.6H_2O$ (20 mM), and acetate buffer (300 mM, pH 3.6) in a ratio of 10:1:1 v/v/v were prepared. The methanol extract (FR) and each column chromatographic fraction (I-VI) dissolved in DMSO (1 mg/mL) served as sample solutions. In a 96-well plate, 10 µL aliquot of the sample solution were diluted with 300 µL FRAP reagent at room temperature for 30 minutes. The value of dilution factors was obtained by reading the plate on a Multiskan Spectrum Plate Reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 593 nm. Using L-Ascorbic acid at concentrations ranging from 0 to 1000 M as a control, the dilution factors for higher concentrations of the extract (FR) and fractions were obtained to calculate the FRAP in µM ascorbic acid equivalents per gram (µM AAE/g) of dry weight of the extract.

Determination of Trolox Equivalent Absorbance Capacity (TEAC)
Procedures of Re et al. were used to evaluate the methanolic extract of *Chromolaena odorata* and its column chromatographic fractions (I-VI) for total antioxidant activity.[18] A working solution containing 88 µL of potassium-peroxodisulfate ($K_2S_2O_8$) (140 mM) and 5mL of ABTS (3-ethylbenzo-thiazoline-6-sulfonic acid) (7 mM) (Merck, South Africa) was prepared in the dark and left for 24 hours at room temperature. The methanol extract (FR) and each fraction (I-VI) dissolved in DMSO (1 mg/mL) served as the sample solution. Varying concentrations (between 0 and 500 µM) of Trolox (6-hydrox-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as standard. The absorbance (~ 2.0 ± 0.1) of diluted working solution and ethanol was used obtained as start-up (control). In a 96-well plate, 25 µL of sample solution was diluted with working solution (300 µL) in the dark and left for 30 minutes at room temperature. With a Plate Reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 734 nm wavelength (25 °C), the TEAC of each test solution was obtained and expressed in µM Trolox equivalents per gram (µM TE/g) of dry weight of the extract.

Determination of Oxygen Radicals Absorbance Capacity (ORAC)
The method of Prior et al. was adopted with slight modification, to determine the ORAC of the methanolic extract of *Chromolaena odorata*.[19] A working solution of 138 µL fluorescein sodium salt and 12 µL of the extract and each fraction in DMSO (1 mg/mL) was separately prepared in an illuminated 96-well plate, in triplicate. A 50 µL of AAPH (in 6 mL Phosphate buffer) (150 mg/mL) was mixed with each test solution in a 96-well plate. With a Fluoroskan Spectrum Plate Reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 485 nm excitation wavelength and 530 nm emission wavelength, the absorbance of each plate was determined. A calibration curve with R^2 = 0.9514 was generated from varying concentrations (range from 83 to 417 µM) of trolox stock solution.
The value of ORAC (µMTE/g) was determined by the regression equation below:

$$Trolox\ concentration\ (Y\ in\ \mu M) = a + bX + cX^2 \quad \text{.....I}$$

Y = Concentration of Trolox (µM)
X = Net area (under Fluorescence Decay Curve)

Lipid Peroxidation (LPO) Assay
Using a slightly modified approach, the method of Snijman et al. was adopted to estimate the Fe (II)-induced microsomal lipid peroxidation (LPO) activity of *Chromolaena odorata* methanol extract.[20] To prepare the TCA reagent (Solution A), 0.125 mL of BHT solution containing 0.8 g of butylated hydroxytoluene and 10 mL of ethanol was diluted with 100 mL of TCA-EDTA solution (containing tetrachloroaceticic acid (10 g), EDTA (29.2 mg), and distilled water (100 mL). Also, 0.67% TBA solution (containing thiobarbituric acid (0.67 g)) and distilled water (100 mL), $FeSO_4$ solution (containing $FeSO_47H_2O$ (139 mg)) and pure water (200 mL), and a buffer solution (KCl (1.15 g)), K_2HPO_4 (1.7418 g), KH_2PO_4 (1.3699 g), and distilled water (100 mL) were prepared. A 0.5mL microsome solution in 0.5mL KCl-buffer was prepared in a 1 mL container as microsome blank, and extract and EGCG in DMSO (1 mg/mL) separately served as the sample solution and positive control solution[20]. An aliquot (50 µL) of each of the test samples and control solution was separately diluted with 300 µL microsomes and incubated at 37°C for 30 minutes in a shaking water bath. Thereafter, 100 µL each of $FeSO_4$-solution and KCl-buffer was mixed with the solutions and left for 1 hour in a shaking water bath at 37°C, before adding 1 mL of TCA reagent. The resultant solution in each tube was vortexed and centrifuged for 15 minutes at 2000 rpm, before transferring 1 mL of the supernatant to a fresh test tube containing 1 mL of 0.67% TBA solution. The final solution was also vortexed and heated for 20 minutes in a water bath at 90°C. The absorbance of each resulting solution was determined with a plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 532 nm and the percentage inhibition was determined by the following:

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$$\%\ Inhibition = \frac{Acontrol1 - Asample}{Acontrol2}\ x\ 100$$
……II
Acontrol1 = Value obtained for positive control
Acontrol2 = Value obtained for microsome solution
Asample = Value obtained for sample solutions

Determination of Anti-Tyrosinase Activity
A previously reported approach by Champo et al. was adopted with slight modifications to evaluate the anti-tyrosinase activity of *Chromolaena odorata* methanol extract.[21] The sample solution (1 mg/mL of the extract in DMSO) was diluted with 50 mM Na_3PO_4 (pH 6.5) into six concentrations ranging from 1000 µg/mL to 31.25 µL. A control solution of kojic acid and DMSO (1 mg/mL (w/v)) was also diluted into six concentrations. Using a 96-well plate, an aliquot (70 µL) of each concentration of sample solution and control solution was separately dispensed in triplicate. For both the sample and control solution, 30 µL of buffered tyrosinase (from mushroom) (250 units/mL) was mixed with the solutions in each well and left for 5 minutes at room temperature. Thereafter, 110 µL of L-tyrosine (substrate) (2 mM) was added and incubated at room temperature for 30 minutes. Using a plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 490 nm, absorbance was determined to calculate the percentage tyrosinase inhibition using the following formular:

$$Tyrosinase\ inhibition\ (\%) = \frac{(A-B)-(C-D)}{(A-B)}\ x\ 100$$
……III

A = Value of the control with enzyme; B = Value of the control without enzyme;
C = Value of sample with enzyme; D = Value of sample without enzyme.

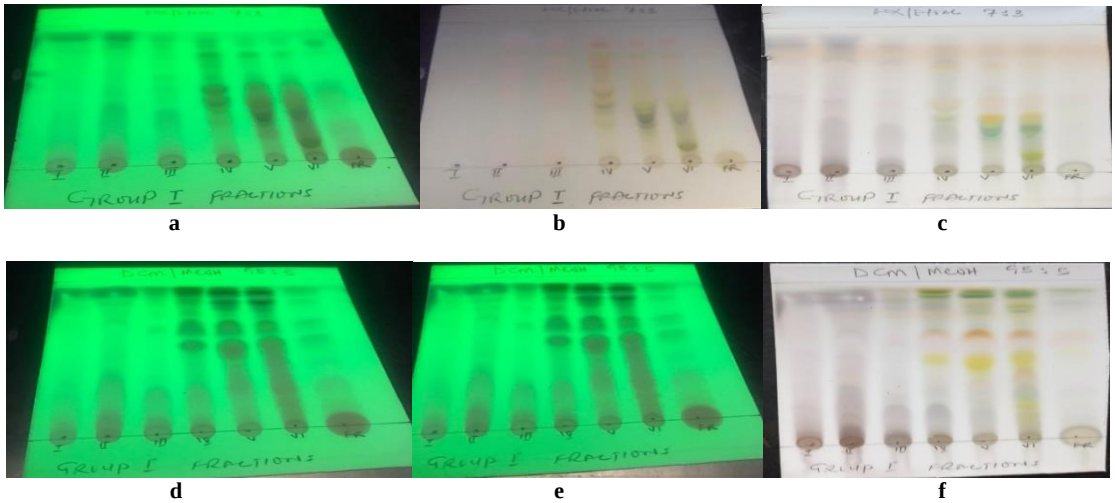
Determination of Anti-Elastase Inhibition
The anti-elastase activity of *Chromolaena odorata* methanol extract was determined by amount of p-nitroanilide released from the substrate (N-succ-$(Ala)_3$-nitroanilide (SANA)).[21] In a 96-Well plate, 200 µL aliquot of SANA (1 mM) in tris-HCl buffer (0.1 M) (pH 8.0) was vortexed mixed with 20 µL of sample solution containing the extract (FR) in tris-HCl (1 mg/mL (w/v)), in triplicate. Same volume of sample solution was separately mixed with Tris-HCl buffer and oleanolic acid as control and positive control respectively. The mixtures in all the wells were pre-incubated for 10 minutes at 25 ^{0}C. A aliquot (40 µL) of elastase (from porcine pancreas) in tris-HCl buffer (0.03 Units/mL) was added to the solution and further incubated for 10 minutes at room temperature. The absorbance at 410 nm was determined with plate reader (Thermo Fisher Scientific, Waltham, Massachusetts, USA) to calculate the percentage elastase inhibition with the following formular:

$$Elastase\ inhibition\ (\%) = \frac{1-B}{A}\ x\ 100$$
……IV
A = Activity of enzyme activity without sample
B = Activity of enzyme with sample

Statistical Analysis
The data obtained from the experiments (in triplicate) were presented in mean ± standard deviation (SD). The values recorded for FRAP, TEAC, and ORAC were corresponded with their respective templates. The results of TRY and ELA inhibitory activities, and LPO assays reported in percentage inhibitions ± SD.

Results and Discussion

Thin Layer Chromatographic Plates Profiling
According to Navarro-Blasco and González-Paramás,[2] ethnomedicinal procedures used in traditional ways may be used to investigate some effective adaptability of various medications and determine their active component. In this work, we chose *C. odorata* as a medicinal plant based on ethnomedicinal applications and extracted it for TLC profiling before spraying it with Vanillin-sulphuric acid reagent. The result of the TLC developed in the study (Figure 1) establishes that due to the weaker polarity of the solvents, spots are noticed in the bottom level of the TLC plate after observing in short UV (254nm) revealing filthy green colour. At a long UV (366nm), the first three spots (I, II, III) and the crude extract (FR) spots were not readily identifiable. The stains are somewhat clearer after being sprayed with sulfuric Vanillin and heated. After examining in short UV (254nm) and long UV (366nm), spots are noticed in the top layer of the TLC plate due to the higher polarity of solvents. The dots at the upper layer are quite visible after being sprayed with Vanillin sulphuric and heated. TLC provides several benefits, including cheaper costs, faster analysis, the ability to do numerous detections, and specialized derivatization on the same plate. TLC profiling of 100% hexane extract is accurate for each plant species based on the separation pattern, i.e., inferred with the termination based on the ratio of solvent systems consisting of hexane: ethyl acetate and dichloromethane: methanol, followed by detection using various detection systems[22]. Based on the TLC profiling, after observing the location and color of the spots in both solvent systems, majority of the extracts exhibited phenols, saccharides, flavonoids, tannins, and other bioactive compounds.



a **b** **c**

d **e** **f**

Figure 1: TLC plate under 254 nm with SSA (a), SSB (d)**;** TLC plate under 366 nm with SSA (b), SSB (e); TLC plate with SSA when sprayed with Vanillin sulphuric and heated (c), SSB (f)

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Inhibitory activity on skin enzymes

Total antioxidant potentials, inhibitory activities of skin enzymes, and ultraviolet spectral properties of the methanolic extract and column chromatographic fractions of *Chromolaena odorata* have all been evaluated in this study, in vitro. This has provided important new information about the potential applications of this plant in dermatology and cosmetics. The findings of our investigation have a substantial impact on our knowledge of *Chromolaena odorata* bioactive characteristics. According to the result in Table 1, *Chromolaena odorata* methanol extract significantly inhibits important skin enzymes that are involved in hyperpigmentation, aging, and wrinkles. The noteworthy inhibition of tyrosinase, elastase, and collagenase suggests that *Chromolaena odorata* may be a good source of naturally occurring substances having advantageous effects on the skin enzymes. These findings correlate with those of other studies on natural compounds derived from medicinal plants, which have demonstrated encouraging enzyme inhibitory properties that may have consequences for formulations used in skincare products.[2] The enzyme tyrosinase is primarily found in plants, animals, and microbes and is essential to the process of melanogenesis. A significant rate-limiting enzyme that catalyzes the synthesis of melanin and browning of enzymes is tyrosinase. Tyrosinase inhibitors have been frequently utilized in cosmetics for skin whitening.[24] Kojic acid is a fungus metabolite that has tyrosinase-inhibiting action. The ability of kojic acid to chelate copper at the active site has been linked to its inhibitory actions in this situation. As a result, kojic acid is frequently employed as a positive control for tyrosinase inhibitors. Anti-tyrosinase activity of *C. odorata* methanol extracts was evaluated with kojic acid as a reference molecule and L-DOPA and L-tyrosine as substrates and (Table 1). *C. odorata* was shown to have some element of tyrosinase inhibitory activity. However, epigallocatechin gallate (EGCG) inhibits lipid peroxidation (LPO), but not tyrosinase or elastase. The inhibitory impact of oleanolic acid (OLEAN) on *C. odorata* extract is null. It is possible that EGCG, to some extent, protects organs or tissues from a variety of disorders. Furthermore, EGCG promotes osteogenesis.[25,26] Although EGCG research is still in its early stages and is fraught with controversy, EGCG appears to be helpful to health.

Antioxidant activity

Overall, methanol extract and fractions of *C. odorata* in this work demonstrated a significant antioxidant potential in addition to their inhibitory effects against skin enzymes. Table 2 shows the overall antioxidant capacities of *C. odorata* methanol extract, including FRAP, TEAC, and ORAC. With significant difference at $p > 0.05$ confidential limit, ORAC has the greatest value (in Figure 2), followed by TEAC, while FRAP has the lowest value (Figure 2). EGCG, on the other hand, has around 37.1% FRAP, 37.2% TEAC, and 27.2% ORAC, respectively, but the difference was not significant when compared to FR, which has approximately 23.8% TEAC, 20.0% ORAC, and 18.9% FRAP in the entire extract. However, the methanol extracts were not noticeable in samples I through V, except for sample VI, which was likewise much higher. The existence of powerful antioxidants was established in the majority of the plant extracts. The presence of bioactive compounds with free radical scavenging abilities is suggested by the antioxidant capacities observed in these samples. These compounds are essential for providing skin protection against oxidative stress-induced damage linked to skin aging and other dermatological conditions[3]. *Chromolaena odorata* has significant antioxidant potential, which is in line with the growing interest in using naturally occurring antioxidants from medical plants for cosmetic applications.

The process of antioxidant production is essential to human health. The molecular nature of EGCG allows us to classify it as an antioxidant. The phenol rings in the EGCG structure prevent it from producing reactive oxygen species that serve as electron trap and scavengers to free radicals,[27,28] and lessen the damage that oxidative stress causes.[29] According to reports, EGCG can effectively prevent blood platelet-induced oxidative stress-induced protein tyrosine nitration,[30] and because it is an antioxidant, it can enhance mitochondrial activity.[31] High EGCG concentrations, however, have been linked to prooxidation and self-oxidation indicated by the release of cytotoxic compounds.[32-34] Chen et al. for instance, found that during self-oxidation, EGCG and EGC generate catechol-quinone that interact with erythrocyte membrane proteins, which results to membrane protein aggregates. Meanwhile, at physiological concentrations between 1-2 M and 10 M, reactive oxygen species released by EGCG in trace level, activate some signal pathways and induce the defense systems in cells, principally displaying its antioxidant benefits. Based on results,[32-35] it only prevents lipid peroxidation (LPO). Also, EGCG's metabolic products may be linked to its intricate biological effects.[36-38]

UV-Spectrum properties

Furthermore, the determining the UV-spectrum properties of methanolic extract and fractions of our plant has revealed the potential of *Chromolaena odorata* as UV absorbers. UV irradiation of the various spots on the A, B, and C TLC plates reveals that each of the three fractions contains at least one spot with orange colour when seen under UV light at 366 nm, which occurs to be VI without inhibition at absorbance 4.001 (Table 3). The single isolated chemical B (dichlorometane: methanol) from the most active fraction (DCF) contains absorption bands that match to flavonoids [max: 280, 285, 295, 300, 305, and 310 nm]. The UV- spectra for A and C (Table 3) in this study corroborate with previous findings.[4] The potential of plant-derived chemicals to absorb UV light is particularly intriguing when it comes to cosmetics, as prevention of UV-induced photodamage is crucial. Our research sheds important light on the potential of *Chromolaena odorata* for photoprotective qualities and its use in the creation of natural sunscreen formulas.[5]

Table 1: Skin Enzymes and Lipid Inhibitory Activities

S/N	Samples	LPO (µg/mL)	TYR (µg/mL)	ELA (µg/mL)
1	FR	99.50	173.125	331.50
2	I	500	500	500
3	II	500	500	500
4	III	365.50	413.750	500
5	IV	360.00	348.125	500
6	V	153.50	273.750	500
7	VI	117.50	234.375	500
8	EGCG	77.00	NA	NA
9	KJA	NA	9.150	NA
10	OLEAN	NA	NA	14.50

FR: methanol extract of *Chromolaena odorata*; **LPO**: lipid peroxidation; **TYR**: tyrosinase; **ELA**: elastase; **EGCG**: epigallocatechin gallate; **KJA**: kojic acid; **OLEAN**: oleanolic acid; **NA**: not applicable

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Table 2: Total Antioxidant Capacities

S/N	Samples	FRAP (µMAAE/g)	TEAC (µMTE/g)	ORAC (µMTE/g)
1	FR	311.74 ± 2.41	798.25 ± 1.18	1491.28 ± 2.30
2	I	34.00 ± 1.89	11.20 ± 4.71	405.12 ± 0.92
3	II	39.52 ± 1.32	25.95 ± 0.78	139.13 ± 1.08
4	III	75.01 ± 3.18	73.00 ± 2.77	65.11 ± 3.21
5	IV	115.43 ± 1.53	97.05 ± 2.35	779.27 ± 3.29
6	V	163.19 ± 1.59	498.82 ± 1.15	969.43 ± 3.44
7	VI	298.81 ± 1.43	602.99 ± 2.80	1571.94 ± 1.97
8	EGCG	611.38 ± 1.65	1248.16 ± 3.71	2023.33 ± 0.54

FR: methanol extract of *Chromolaena odorata*; **FRAP:** Ferric-ion Reducing Antioxidant Power Assay; TEAC: Trolox Equivalent Absorbance Capacity Assay; **EGCG**: epigallocatechin gallate.

Table 3: UV Absorption Spectral Characteristics and Photoprotection Activity

S/N	Column Fraction	UV sample (500 µg/mL)	UV absorption region		
			UVA (nm)	UVB (nm)	UVC (nm)
1		FR	250 (2.043)	280 (1.449)	360 (3.332)
	FR	FR + sodium acetate	230 (2.265)	280 (2.606)	360 (2.061)
		FR + boric acid	230 (1.728)	280 (1.614)	360 (1.885)
		I	250 (1.876)	-	-
2	I	I + sodium acetate	230 (2.310)	-	-
		I + boric acid	230 (2.045)	-	-
3		II	250 (1.953)	280 (2.944)	-
	II	II + sodium acetate	230 (2.576)	280 (1.358)	-
		II + boric acid	230 (2.314)	280 (0.969)	-
4		III	250 (2.404)	305 (3.054)	-
	III	III + sodium acetate	230 (2.420)	285 (1.363)	-
		III + boric acid	230 (2.438)	285 (1.546)	-
5		IV	245 (2.369)	295 (2.997)	-
	IV	IV + sodium acetate	250 (2.832)	310 (3.192)	-
		IV + boric acid	250 (3.120)	295 (3.680)	-
6	V	V	250 (2.518)	290 (2.528)	360 (1.578)
		V + sodium acetate	240 (2.540)	290 (2.751)	360 (1.698)
		V + boric acid	240 (2.552)	290 (3.098)	360 (2.236)
7		VI	250 (2.483)	300 (2.919)	360 (4.001)
	VI	VI + sodium acetate	250 (2.993)	300 (3.296)	360 (3.332)
		VI + boric acid	250 (1.728)	300 (3.271)	360 (2.912)

Conclusion

Based on total antioxidant potentials, skin enzyme inhibitory activities, and ultraviolet spectral characteristics, our study is a piece of strong evidence that *Chromolaena odorata* methanol extract and its column chromatographic fractions are promising source of essential bioactive compounds with dermatological and cosmeceutical relevance. The results of this investigation call for more research, including in vivo investigations and formulation development, to fully utilize the medicinal and skincare advantages of *Chromolaena odorata* for real-world uses in the wellness and cosmetics sector.

Conflict of Interest

The authors declare no conflict of interest.

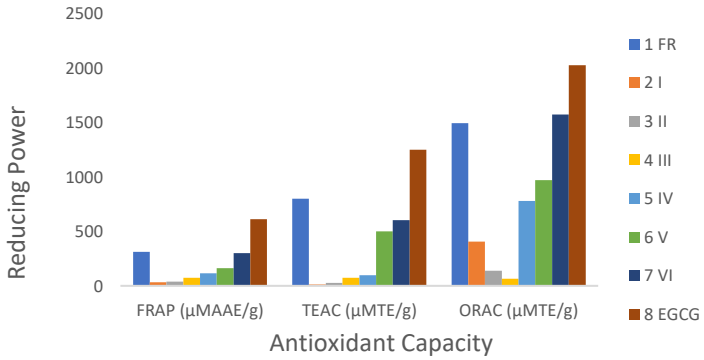

Figure 2: The statistical chart of antioxidant capacities of *C. odorata*

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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