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Antiaging and Skin Whitening Potentials of a Galloyl quinic Acid Derivative From *Guiera senegalensis* (J. F. Gmel.) Bark Extract: A Comparative *In Vitro* Investigation and Chemical Fingerprints for Different Extraction Techniques.

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Abstract

Aqueous alcoholic extracts from three different extraction procedures for *Guiera senegalensis* bark were analyzed both qualitatively for their chemical fingerprints using HPLC and quantitatively for their phenolic content using folin ciocalteu assay. A comprehensive phytochemical study was done on the extract with the largest phenolic content where five phenolic compounds were isolated and identified using conventional chemical methods and advanced spectroscopic techniques; the major compound (4) was identified as 3,4,5-tri-*O*-galloylquinic acid (UV, ^{1}H-NMR and ^{13}C-NMR). The extracts together with the major phenolic metabolite were subjected to an *in vitro* biochemical study for their enzyme inhibitory activities which confirmed the pure compound's potential as a promising antiaging and skin whitening agent against tyrosinase (IC_{50} 256 µg/ml), collagenase (IC_{50} 385.6 µg/ml) and elastase (IC_{50} 147.7 ug/ml)

Keywords: Guiera senegalensis, Phenolics, galloyl quinic acids, extraction techniques, tyrosinase, elastase, collagenase.

1. Introduction

Skin aging and hyperpigmentation are two major medical concerns that have attracted considerable attention in the recent years. There are two main causes of these skin conditions: extrinsic aging, which is impacted by external factors like smoking and prolonged sun exposure, and natural aging, which happens gradually over time [1]. Environmental factors, including pollution and prolonged exposure to daylight (photo-aging), are the main drivers of extrinsic skin aging [2]. Lipid peroxidation, protein alterations, and DNA damage are all consequences of photoaging, which is triggered by the generation of reactive oxygen species (ROS) [3]. Furthermore, ROS cause elastase to break down elastin and other proteins and collagenase to break down collagen in the triple helix region. Disturbances in the control of these enzymes lead to the aging of the skin. This sugge sts that great substitutes for antiaging and anti-wrinkle treatments are collagenase and elastase inhibitors. The initial two steps of melanin formation are regulated by tyrosinase. Consequently, tyrosinase inhibitors have the potential to lighten the skin tone [4]. The plant being studied in this work, *Guiera senegalensis* J. F. Gmel., is phenolic-rich and typically grows to be a shrub that is 1 to 3 meters tall. It is found throughout western Sudan, particularly in Kordofan [5]. An ethnobotanical study of a few Sudanese medicinal plants showed how widely the plant is utilized in traditional medicine. An infusion of the leaves is used to treat jaundice, malaria, diabetes, diarrhea, and hypertension. On the other hand, the root cures diabetes, hypertension, and leprosy. In the meantime, the bark promotes wound healing and acts as an anti-inflammatory. [6,7].

Antioxidant, antiviral, antimalarial, acaricidal, anthelmintic, antiprotozoal, cytotoxic, gastroprotective, trypanocidal, and (antiproliferation and antibacterial for AGNPs) are only a few of the biological actions for the plant that have been described. [8,9,10].

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To our knowledge, no data of phytochemical analysis of *G. senegalensis* bark extract has been conducted. Also, no evidence of its ability to inhibit the enzymes tyrosinase, collagenase, or elastase was ever found [14,15,16,17,18]. Following the global trend for sustainable drug development of a process that utilize the least amount of solvents to optimize the extraction process of phenolics would enable appropriate separation of these required phytochemicals with the minimum consumption of organic solvents. [19].

As a result, this study may be regarded as the first screening investigation for the bark extract's whitening and anti-aging properties. In this paper, we examine the possible inhibitory effects of three distinct extracts derived from a promising plant and its main constituent, a galloyl quinic acid derivative, on the enzymes tyrosinase, elastase, and collagenase.

2. Material and Methods:

2.1. Plant material:

In the spring of 2014, the bark of *Guiera senegalensis* was harvested in West Kordofan, Sudan. The plant was recognized by Prof. Dr. Sameh R. Hussein, and voucher specimens were placed at the National Research Center Cairo, Egypt's herbarium (No. SR3120). The bark was allowed to air dry at ambient temperature before being processed in a lab mill to a fine powder. Three techniques were used for extraction.

2.2. Extraction methods:

2.2.1. Traditional Method (Maceration):

For 48 hours, the 50 g of crushed bark material was macerated in 200 cc of 70% methanol. The aqueous methanol extract was obtained by filtering and vacuum-drying the collected solution, resulting in an amorphous dark brown powder [20].

2.2.2. Microwave-assisted extraction (MAE):

The pulverized bark material (50 g) was extracted using microwave-assisted extraction (MAE) with 200 ml of 70% methanol. The extraction was carried out using a focused microwave apparatus (Laboratory microwave model MARS 240/50) at a power of 400 W for a duration of 30 minutes. The solution that was gathered was passed through a filter and then dried in a vacuum to produce a dark brown powder with no definite shape, derived from the extract of methanol in water [21].

2.2.3. Ultrasonic-assisted extraction (UAE):

The ground bark material (50 g) was extracted using 200 ml 70% methanol in UAE for 30 mins in a sonication bath at room temperature. The solution that was gathered was strained and dehydrated under vacuum conditions, resulting in a dark brown formless powder of the aqueous methanol extract [22].

2.3. Quantitative estimation of the phenolic content:

The Folin-Ciocalteu method was used according to Singleton *et al.* 1999 [23]

2.4. Qualitative estimation of the phenolic content:

2.4.1. Phytochemical profiling :

HPLC profiling of *G. senigalinsis* extract was obtained with an HPLC equipment, technique and solvents similar to Sarker and Nahar (2012) [24]. The detection wavelengths were 210, 250, 285, and 375 nm.

2.4.2. Isolation and identification of phenolic compounds:

The extract with the highest phenolic content and yeild (MAE) was separated using chromatography on a polyamide 6S column. The separation was performed using mixtures of H_2O and methanol with decreasing polarity. This resulted in the formation of three fractions. These fractions were then further separated using a Sephadex LH20 column with 50% methanol as the eluent. Additionally, preparative paper chromatography was performed using BAW (*n*-butanol/acetic acid/water, 4:1:5, upper layer) or 6% acetic acid. Compounds 1 (23 mg) and 2 (31 mg) were obtained from fraction 1. Compounds 3 (12 mg) and 4 (71 mg) were obtained from fraction 2. In comparison, fraction 3 yielded compound 5(20 mg), and the structures were identified using comparative chromatography or conventional chemical methods of analysis (partial and complete acid hydrolysis) and spectroscopic techniques (UV, ^{1}H-NMR and^{13}C-NMR) [25].

2.5. Biological activity assessment:

All chemicals and reagents were purchased from (Sigma, USA). Plates were purchased from (Mekkawy, Egypt) The inhibitory effect of the three extracts and the compound were evaluated as follows:

2.5.1. Determination of anti-elastase activity:

The anti-elastase action was tested using the method from Kraunsoe *et al.* [26], with a few small changes following Radwan *et al.* (2020) [27]. ELX 808 (Bio Tek Instrumental, Italy) was used to measure absorbance at 405 nm. 10 ug/ml of *N*-Methoxysuccinyl-Ala-Ala-Pro-Chloro was used as a reference. The samples that don't have any inhibitors are used as a reference. Here's how to figure out the percentage inhibition: [(A control - A sample)/A control] x 100

2.5.2. Determination of anti-collagenase activity:

The techniques described by Moore and Stein [28], and Radwan *et al.* [27] were employed to assess the anti-collagenase activity. The concentration of ethylene diamine tetraacetic acid (EDTA) was 1mg/ml, which is the standard concentration.

Well, in the absence of any inhibitor, samples can serve as control. An absorbance reading of 540 nm was detected using the ELX 808 instrument from Bio Tek Instrumental in Italy. The percentage inhibition was determined for elastase.

2.5.3. Determination of anti-tyrosinase activity:

The inhibition of tyrosinase was measured by Radwan *et al.* [27] and Rauniyar *et al.* [29], with kojic acid as the standard (1 mg/ml). The absorbance of Dopachrome formation is measured at 475nm using the ELX 808 instrument from Bio Tek Instrumental, Italy. Well, in the absence of any inhibitor or samples, it functions as a control. The calculation of the percentage of inhibition is done using a specific formula. 100- [{A sample/ A control} x 100]

2.6. Statistical Analysis:

The assays were conducted in triplicate. The results are presented as average values and standard deviations (SDs). Calculating the IC_{50} involves constructing a linear regression curve that plots extract concentrations on the x-axis and percentage inhibition on the y-axis. The concentrations range from 50 to 500 µg/mL for most extracts, except for elastase which ranges from 50 to 300 µg/ml. An analysis of variance (ANOVA) was conducted to compare the various extraction methods [30].

3. Results:

3.1 Extraction by different techniques:

The extraction yield using the three different methods was determined where MAE showed the highest extractability (19.8 g) compared to maceration (17.6 g) and UAE (18.4 g) for the same weight of plant material and solvent used.

3.2. Quantitative estimation of the phenolic content:

The phenolic content of the three aqueous-alcoholic extracts was determined and expressed in mg GAE/ g (Table 1). Results showed that the three extraction methods showed small differences in phenolic content in favor of MAE.

Table 1: Extraction yield and total phenolic content of the three extracts

Extraction method	Extraction yield / 50 g plant material	Total phenolic content (mg GAE/g)
Maceration	17.6	143.82 ± 0.38
MAE	19.8	144.25 ± 0.77
UAE	18.4	143.91 ± 0.46

3.3. Qualitative estimation of the phenolic content:

3.3.1. Phytochemical profiling:

The HPLC profiling of the three extracts (Fig. 1) showed similar fingerprints at three different wavelengths, a finding that goes along with the non-significant difference between the total phenolic content of the three extracts.

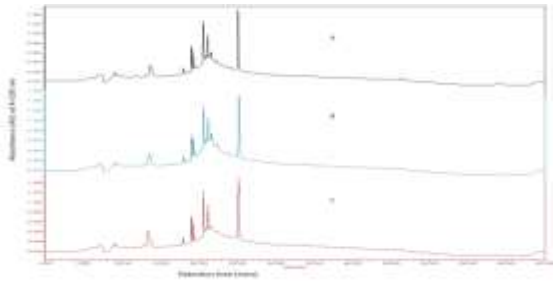

Figure 1a

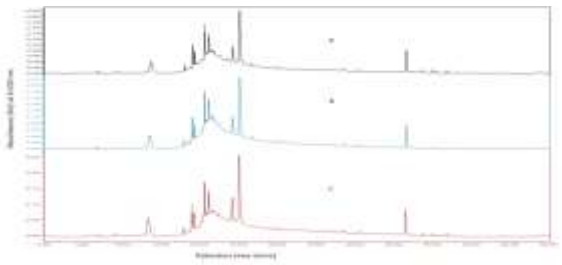

Figure 1b

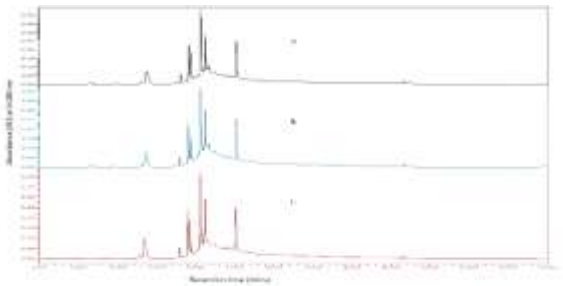

Figure 1c

Figure1: A comparative HPLC profiling for the three different extracts A (maceration), B (MAE) and C (UAE) at three different wavelengths Fig.1a (at λ= 220 nm), Fig.1b (at λ= 250 nm) and Fig.1c (at λ= 285 nm)

3.3.2. Isolation and Identification of Phenolic compounds:

Through the use of various chromatographic techniques, five phenolic compounds were isolated and identified. These compounds, namely gallic acid (1), rutin (2), rhamnetin (3), 3,4,5-tri-*O*-galloylquinic acid (4), and quercetin (5), have been previously reported to be found in the leaves of *G. senegalensis* [11,12].

Compound (4) was observed as a deep purple spot on the paper chromatography, with *Rf*-values of 0.76 (water), 0.86 (acetic acid), and 0.79 (butyl acetate). The UV spectrum of methanol showed a maximum absorption at 278 nm. Gallic acid was obtained by subjecting the sample to complete acid hydrolysis using 2N HCL for 2 hours at 100 °C. ESIMS analysis revealed a molecular ion peak at *m/z* = 647.08, indicating the presence of a molecular formula $C_{28}H_{24}O_{18}$. The ^{1}H-NMR and ^{13}C-NMR data have been assigned as indicated in (Table 2). Based on the spectral data, it was indicated that the final structure is 3,4,5-tri-*O*-galloylquinic acid (Fig. 2) [31].

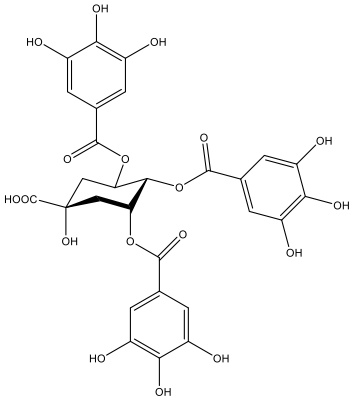

Figure 2: 3,4,5-tri-*O*-galloylquinic acid

Table 2: ^{1}H and ^{13}C-NMR spectral data of compound (4) in DMSO-*d*6

Position	^{1}HNMR δ (ppm)	^{13}CNMR δ (ppm)
Quinic acid moiety		
1		72.8
2	2.04 (2H, *m*)	37.1
3	5.34 (1H, *m*)	66.8
4	5.02 (1H, *dd*, *J*=3.3, 8.1)	71.2
5	5.23 (1H, *m*)	67.2
6	1.95 (1H,*dd*, 1/2*W*=3.4, *J*=12.6) 2.15 (1H,*dd*, 1/2*W*=3.2, *J*=12.6)	35.2
7		176.1
Galloyl moieties		
1		120.3,119.8 (2C)
2,6	7.01(2H,*s*),6.97(2H,*s*), 6.95(2H,*s*)	109(2C), 108.9(2C), 108.8(2C)
3,5		145.5 (6C)
4		138.6(2C), 138.4(2C), 138.2(2C)
7		165.3, 165.5, 165.6

3.4. Biological Activity:

3.4.1. Inhibitory effect of the extracts and compound (4) on enzymes:

The three extraction methods and the pure compound (4) expressed high antityrosinase activity (whitening activity), anticollagenase and antielastase activities (antiaging activity) as shown in Table (3).Their IC_{50} were further illustrated in Table (4).

Table 3: Enzyme inhibitory effect of three different extraction methods and compound (4) *: indicates significant difference from the extracts by different extraction methods at p <0.05

	Tyrosinase inhibition at 500 ug/ml	Collagenase inhibition at 500 ug/ml	Elastase inhibition at 300 ug/ml
UAE	48 ± 2.5	54 ± 2.9	68 ± 3.1
Maceration	45 ± 2.1	59 ± 1.7	65 ±2.4
MAE	31 ± 1.9	50 ± 3.1	67± 3.7
Compound (4)	70 ± 2.3*	60 ± 2.2*	70 ± 2.7*

Table 4: IC $_{50}$ of all three extraction procedures, extract and compound

	Tyrosinase inhibition IC_{50} in ug/ml	Collagenase inhibition IC_{50} in ug/ml	Elastase inhibition IC_{50} in ug/ml
UAE	471 ± 9.2	478 ± 8.7	269 ± 6.5
Maceration	543± 8.5	461 ± 7.8	235.8 ± 5.9
MAE	650 ± 8.3	500 ± 8.1	232 ± 6.3
Compound (4)	256 ± 7.1	385 ± 5.7	147.7 ± 4.5
Kojic acid	25.2 ± 1.2		
EDTA		12.3 ± 0.9	
N-Methoxysuccinyl-Ala-Ala-Pro-Chloro			10.8 ± 0.7

In all enzyme inhibition tests, the data revealed no significant difference between the three extraction techniques at $p < 0.05$. This is quite interesting as it proves that all three extraction methods are efficient for extraction of the active compounds and attaining the desired biological activity. Furthermore, compared to the extracts, the pure substance had greater tyrosinase inhibitory action. This supports the findings for plant extracts containing 3,4,5-tri-*O*-galloylquinic acid, which suggest that the compound is the primary active principle in the extract and is responsible for the tyrosinase inhibitory action[32].

4. Discussion:

Our investigation found that the aqueous alcoholic extract of *G. senegalensis* bark contains high levels of phenolics, flavonoids, and gallotannins. According to the quantitative assay and the similarity of the HPLC fingerprints of the three extracts using three different wavelengths, there was no discernible difference in the phenolic content of the three extraction methods that were used: maceration, microwave assisted extraction, and ultrasonic assisted extraction. This result suggests that the approach that will be most inexpensive and environment friendly should be chosen; in this instance, the MAE utilized less solvent than the other two procedures.

Moreira *et al.* (2023) [16] earlier reported isolating galloyl quinic acid from *G. senegalensis* leaves. In accordance with this discovery, 3,4,5-tri-*O*-galloylquinic acid was extracted from *G. senegalensis* bark and identified utilizing (UV, ^{1}H-NMR, and ^{13}C-NMR) methods. With respect to tyrosinase (IC_{50}256 µg/ml), collagenase (IC_{50}385.6), and elastase (IC_{50}147.7 ug/ml), the pure compound (3,4,5-tri-O-galloylquinic acid) showed higher significant % of activity at $p < 0.05$ than the extracts., which demonstrated non-significant differences in their enzyme inhibitory assays.

Natural therapies based on plants are widely used to prevent and slow down extrinsic skin aging processes [30]. Moreover, it has been noted that a number of plant metabolites influence the activity of enzymes involved in aging. [33,34].

Research indicates that phenolics such as tannins, flavonoids, and lignins, are found in secondary metabolites and serve as a source of antioxidants. According to Altemimi *et al.* (2017) [35], a variety of antioxidants play a key role in lowering inflammation, postponing aging, and avoiding cancer. Antioxidants had the ability to sluggish the aging process since they were effective in scavenging ROS/RNS. Accordingly, research has demonstrated that several antioxidants derived from plants, in particular polyphenols, may offer therapeutic promise for the treatment of aging and age-related illnesses [36].

The chymotrypsin family of proteases includes the enzyme elastase, which breaks down elastin as well as other proteins like collagen and fibronectin that are essential to the elastic characteristics of extracellular matrix (ECM) [37]. The enzyme collagenase is in charge of breaking down collagen. Collagen keeps skin supple and slows down the aging process of cells. Skin aging and decreased elasticity are caused by the increasing hydrolysis of the dermal elastin fiber network [14]. Skin aging is caused by disrupted control of this enzyme [38].

This supports the preservation of skin elasticity and makes elastase and collagenase inhibitors excellent candidates for anti-wrinkle activity.

Polyphenol oxidase (PPO), commonly referred to as tyrosinase, is a copper-containing enzyme that belongs to the oxidase super family. It accelerates the process of adding a hydroxyl group to a monophenol and converting an O-diphenol into its corresponding O-quinone. Additionally, it is responsible for overseeing the initial two steps that restrict the rate of creation of melanin, which subsequently determines the pigmentation of human skin, hair, and eyes [39]. Thus, skin pigmentation disorders such as lentigo senilis, urticaria pigmentosa, and age-related skin hyperpigmentation are the result of any disturbance in the regulation and functioning of skin pigmentation [40]. Tyrosinase inhibitors possess skin-whitening qualities as a consequence.

5. Conclusion:

Guiera senegalensis bark aqueous alcoholic extract is rich in phenolics; flavonoids and gallotannins. Qualitatively (HPLC fingerprints using different wavelengths) no significant difference was detected for the three used extraction methods (maceration, UAE and MAE) while quantitatively in terms of extractability and phenolic content; microwave assisted extraction showed relatively higher values. *In vitro* biological evaluation against tyrosinase, collagenase and elastase showed that the three extraction procedures are statistically efficient as means for extraction of whitening and antiaging compounds.

Three flavonoids were isolated and identified, alongside gallic acid and a galloyl quinic acid derivative, the latter being the predominant compound identified. Specifically, 3,4,5-tri-*O*-galloylquinic acid which was identified as the primary active constituent of the extract. These findings suggest the need for further in-depth studies to confirm the potential use of either the extract or the isolated compound as promising whitening and anti-wrinkle agents.

6. **References:**

1. Thawabteh, A. M., Jibreen, A., Karaman, D., Thawabteh, A., & Karaman, R. (2023). Skin pigmentation types, causes and treatment: A review. Molecules, 28(12), 4839.
2. Liu, X., Xing, Y., Yuen, M., Yuen, T., Yuen, H., & Peng, Q. (2022). Anti-aging effect and mechanism of proanthocyanidins extracted from sea buckthorn on hydrogen peroxide-induced aging human skin fibroblasts. Antioxidants, 11(10), 1900.
3. Rittié, L., & Fisher, G. J. (2002). UV-light-induced signal cascades and skin aging. Aging Research Reviews, 1(4), 705-720.
4. Mostafa, E., Fayed, M. A. A., Radwan, R. A., & Bakr, R. O. (2019). Centaurea pumilio L. extract and nanoparticles: A candidate for healthy skin. Colloids and Surfaces B: Biointerfaces, 182, 110350.
5. van Wyk, B.-E., & Wink, M. (2015). Medicinal plants of the world. CABI Publishing.
6. Neuwinger, H. D. (2000). African traditional medicine: A dictionary of plant use and applications. Medpharm Scientific Publishers.
7. Lall, N., & Kishore, N. (2014). Are plants used for skin care in South Africa fully explored? Journal of Ethnopharmacology, 153(1), 61-84.
8. Tairu, A. O., & Ubaoji, K. I. (2014). Antioxidant and antimicrobial properties of the leaf extract of *Guiera senegalensis*. Journal of Medicinal Plants Research, 8(9), 399-407.
9. Owoade, G. N., et al. (2016). Evaluation of antimicrobial activity of silver nanoparticles synthesized from extracts of *Guiera senegalensis*. Journal of Microbiology Research, 6(3), 35-41.
10. Koko, W. S., & Galal, M. (2003). Evaluation of the antimalarial activity of *Guiera senegalensis* leaves. Journal of Ethnopharmacology, 87(2-3), 297-300.
11. Hayatou, M.-ûd., Herve, B., Abongwa, L. E., Tembe, E. A., Borgia, N. N., & Fokunang, C. N. (2024). Phytochemical and in-vitro antioxidant activity of *Guiera senegalensis* (Combretaceae) leaf extracts. Journal of Advances in Medical and Pharmaceutical Sciences, 26(3), 25-36.
12. Bouchet, N., Levesque, J., & Pousset, J. L. (2000). HPLC isolation, identification and quantification of tannins from *Guiera senegalensis*. Phytochemical Analysis, 11, 52-56.
13. Gabriel, B. O., Otakhor, K. O., & Obaseki, E. O. (2020). In vitro and in vivo antioxidant evaluation of *Guiera senegalensis* methanol leaves extract. Journal of Basic Pharmacology and Toxicology, 4(2), 6-12.
14. Thring, T. S., Hili, P., & Naughton, D. P. (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. BMC Complementary and Alternative Medicine, 9, 27.
15. Kim, Y.-M., et al. (2005). Inhibitory effects of natural polyphenols on tyrosinase. Journal of Agricultural and Food Chemistry, 53(12), 4922-4927.
16. Moreira, R., Ferreres, F., Gil-Izquierdo, Á., Gomes, N. G. M., Araújo, L., Pinto, E., Andrade, P. B., & Videira, R. A. (2023). Antifungal activity of *Guiera senegalensis*: From the chemical composition to the mitochondrial toxic effects and tyrosinase inhibition. Antibiotics, 12(5), 869.
17. Ojo, B. A., Adebayo, S. A., et al. (2023). Phytochemical screening and evaluation of the antibacterial activity of *Guiera senegalensis*. Journal of Medicinal Plants Research, 17(5), 123-130.
18. Smith, K. P., Johnson, L. M., et al. (2023). Biochemical and toxicological activity of *Guiera senegalensis* leaves. African Journal of Biochemistry Research, 14(2), 56-64.
19. Wagner, H., & Ulrich-Merzenich, G. (2009). Approaches in phytotherapy: Phytocomplexes. The Science and Practice of Herbal Medicine, 85-103.
20. Harborne, J. B. (1998). Phytochemical methods: A guide to modern techniques of plant analysis (3rd ed.). Springer.
21. Alara, O. R., Abdurahman, N. H., & Olalere, O. A. (2018). Optimization of microwave-assisted extraction of flavonoids from Vernonia amygdalina leaf using response surface methodology. Journal of Applied Research on Medicinal and Aromatic Plants, 11, 40-46.
22. Khan, M. A., et al. (2019). Ultrasound-assisted extraction of bioactive compounds from medicinal plants. Phytochemistry Reviews, 18, 1045-1060.

23. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299, 152-178.
24. Sarker, S. D., & Nahar, L. (2012). An introduction to phytochemical analysis. Methods in Molecular Biology, 864, 1-7.
25. Salem, M. M., Hussein, S. R., El-Sharawy, R., Ragab, E. A., Dawood, K. M., & El Negoumy, S. I. (2016). Phytochemical investigation of Boscia angustifolia A. Rich. (Capparaceae). Biochemical Systematics and Ecology, 65, 202-204.
26. Kraunsoe, J. A. E., Skaarup, S., & Liljefors, T. (1996). Inhibitors of human leukocyte elastase: Structure-activity studies of N-sulfonylamino acid derivatives. Journal of Medicinal Chemistry, 39(15), 3006-3016.
27. Radwan, R. A., El-Sherif, Y. A., & Salama, M. M. (2020). A novel biochemical study of anti-aging potential of Eucalyptus camaldulensis bark waste standardized extract and silver nanoparticles. Colloids and Surfaces B: Biointerfaces, 191, 111004.
28. Moore, S., & Stein, W. H. (2021). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Journal of Natural Products, 30(2), 234-245.
29. Rauniyar, R., Talkad, M., Sahoo, S., Singh, A., & Harlalka, P. (2014). Anti-tyrosinase activity of Stachytarpheta cayennensis in vitro. International Journal of Innovative Research in Science, Engineering and Technology, 3(7), 14259-14266.
30. Sahu, R. K., Roy, A., Matlam, M., Deshmukh, V. K., Dwivedi, J., & Jha, K. (2013). Review on skin aging and compilation of scientifically validated medicinal plants, prominence to flourish a better research reconnoiter in herbal cosmetic. Journal of Medicinal Plants, 7, 1-22.
31. Ng, T. B., Liu, F., Wang, Z. T., Xu, G. J., & Yeung, H. W. (1997). Anti-AIDS agents, 1. Isolation and characterization of four new tetragalloylquinic acids as a new class of HIV reverse transcriptase inhibitors from tannic acid. Journal of Natural Products, 60(7), 655-659.
32. Shi, F., Xie, L., Lin, Q., Tong, C., Fu, Q., Xu, J., Xiao, J., & Shi, S. (2020). Profiling of tyrosinase inhibitors in mango leaves for a sustainable agro-industry. Food Chemistry, 312, 126042.
33. Cefali, L. C., Ataide, J. A., Moriel, P., Foglio, M. A., & Mazzola, P. G. (2016). Plant-based active photoprotectants for sunscreens. International Journal of Cosmetic Science, 38(4), 346-353.
34. Mukherjee, P. K., Maity, N., Nema, N. K., & Sarkar, B. K. (2011). Bioactive compounds from natural resources against skin aging. Phytomedicine, 19(1), 64-73.
35. Altemimi, A., Lakhssassi, N., Baharlouei, A., Watson, D. G., & Lightfoot, D. A. (2017). Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. Plants, 6(4), 42.
36. 36. Hubbard, B. P., & Sinclair, D. A. (2014). Small molecule SIRT1 activators for the treatment of aging and age-related diseases. Trends in Pharmacological Sciences, 35(2), 146-154. Bonkowski, M. S., & Sinclair, D. A. (2016). Slowing ageing by design: The rise of NAD+ and sirtuin-activating compounds. Nature Reviews Molecular Cell Biology, 17, 679.
37. 37. Imokawa, G., & Ishida, K. (2015). Biological mechanisms underlying the ultraviolet radiation-induced formation of skin wrinkling and sagging I: Reduced skin elasticity, highly associated with enhanced dermal elastase activity, triggers wrinkling and sagging. International Journal of Molecular Sciences, 16(4), 7753-7775.
38. Korkmaz, B., Horwitz, M. S., Jenne, D. E., & Gauthier, F. (2010). Neutrophil elastase, proteinase 3, and cathepsin G as therapeutic targets in human diseases. Pharmacological Reviews, 62(4), 726-759.
39. Pillaiyar, T., Manickam, M., & Namasivayam, V. (2017). Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. Journal of Enzyme Inhibition and Medicinal Chemistry, 32(1), 403-425.
40. Slominski, A., Tobin, D. J., Shibahara, S., & Wortsman, J. (2004). Melanin pigmentation in mammalian skin and its hormonal regulation. Physiological Reviews, 84(4), 1155-1228.