separations

MDPI

Article

Antioxidant-Activity-Guided Purification and Separation of Octocrylene from *Saussurea heteromalla*

Saboon [1,2], Yamin Bibi [1,*], Samha Al Ayoubi [3], Tayyiba Afzal [1], Sobia Gilani [1], Khafsa Malik [1], Abdul Qayyum [4], Mubashar Hussain [5] and Sunjeet Kumar [6,*]

1 Department of Botany, PMAS-Arid Agriculture University Rawalpindi, Rawalpindi 46300, Pakistan
2 Department of Botany, Women University Mardan, Mardan 23200, Pakistan
3 College of Humanities and Sciences, Prince Sultan University, Riyadh 11586, Saudi Arabia
4 Department of Agronomy, The University of Haripur, Haripur 22620, Pakistan
5 Department of Zoology, University of Gujrat, Gujrat 50700, Pakistan
6 Key Laboratory for Quality Regulation of Tropical Horticultural Crops of Hainan Province, School of Horticulture, Hainan University, Haikou 570228, China
* Correspondence: dryaminbibi@uaar.edu.pk (Y.B.); 184224@hainanu.edu.cn (S.K.)

Abstract: Plants have been a source of medicine since ancient times, and such traditional medications are widely practiced nowadays. *Saussurea heteromalla* is widely used in traditional medicine in the Himalayan region for the treatment of different ailments. However, despite its traditional uses, it is not widely explored for its free radical scavenging abilities and other biological activities. Thus, the current study is aimed at exploring the free radical scavenging ability of *S. heteromalla* extracts, along with the isolation and evaluation of its compound through bioassay-guided purification. From different solvent fractions, an n-hexane extract of a whole plant is found to be most active; thus, it is processed for large-scale extraction and column chromatography. Further, the purification of an active fraction is performed using HPLC–DAD, which led to the isolation of an active peak, identified by GC/MS, as 2-ethylhexyl 2-cyano-3, 3-diphenylprop-2-enoate. This compound, commonly known as octocrylene, is widely recommended for UV-B filter, to be used in the cosmetic industry as photoprotection products. The presence of free radical scavenging activity enhances the sun protection factor of octocrylene. The present study presents the first report on the isolation of this compound from the family Asteraceae. The compound has good free radical scavenging activity and is widely used in the cosmetic industry as a sun protectant and is much less reported from botanical sources.

Keywords: octocrylene; *Saussurea heteromalla*; sun protectant; antioxidants

Citation: Saboon; Bibi, Y.; Ayoubi, S.A.; Afzal, T.; Gilani, S.; Malik, K.; Qayyum, A.; Hussain, M.; Kumar, S. Antioxidant-Activity-Guided Purification and Separation of Octocrylene from *Saussurea heteromalla*. *Separations* **2023**, *10*, 107. https://doi.org/10.3390/separations10020107

Academic Editors: Nguyen Van Quan and Tran Dang Xuan

Received: 20 December 2022
Revised: 20 January 2023
Accepted: 26 January 2023
Published: 3 February 2023

1. Introduction

Plants are natural reservoirs of chemical compounds with majority of them having medicinal values. Approximately 20% of known plant chemicals have been used in pharmaceutical research, giving an optimistic way to the healthcare system for the treatment of chronic diseases [1]. Many important drugs (berberine, ephedrine, reserpine, morphine, aspirin, vinblastine, vincristine, etc.) were discovered and isolated from medicinal plants. The discoveries of industrially important drugs have increased the economic value of medicinal plants and their use in the healthcare system [2].

The identification and isolation of phytochemicals is not a new technique; however, latest methods are better in terms of efficiency of the process. Nowadays, targeted isolation of compounds is preferred, which is based on their bioactivities [3]. Phytochemical compounds possess biological activities due to the presence of various potentially active groups in their molecular structure. Bioassay-guided fractionation and isolation have been the most widely used approach for evidence-based pharmacological in vitro and in vivo studies, where each solvent extract/fraction is investigated for its potential biological

activities. Studies on different solvent extracts/fractions may lead to the identification of novel compounds in the field of pharmaceutical medicine [4].

Phytochemicals exhibit a wide range of properties, including antioxidation. Antioxidants treat certain skin diseases and promote skin health through their scavenging effects. Antioxidants are used as cosmeceuticals in antiaging, anti-acne, and photoprotectant products. To treat skin problems, plant-based cosmetics are being used fluently due to their low side effects. Plants are enriched with antioxidants responsible for reducing free radical activities [5]. Plant-based antioxidant compounds, including quercetin, gallic acid, rutin, and myricetin, have been identified and reported by many researchers [6].

A researcher showed that phytochemicals play an important role in photoprotection against UV-induced skin photodamage through antioxidant and anti-inflammatory properties. Such phytochemicals have been validated to mitigate skin photodamage under in vitro and in vivo conditions through scavenging reactive oxygen species (ROS) and promoting antioxidant defense capacity. Octocrylene is used in sunblocks due to its soothing effect by counteracting radiations and reducing skin damage from sun exposure [7]. The scope of this compound is high and is reported much less from the botanical source. This compound caught our attention because of its ultraviolet (UV) filtering ability to protect the skin from damage due to sun exposure and reduce the harmful effect of ROS. To the best of our knowledge, the present study is the first report on the isolation of octocrylene from the family Asteraceae, showing effective free radical scavenging activity.

Saussurea heteromalla (D.Don) Hand.-Mazz. is a perennial herb, having a height of 60–150 cm, and belongs to the family Asteraceae. Asteraceae is a large plant family with many genera and species and well known for its high medicinal values. The literature revealed several species of this family rich in phenolic compounds with powerful antioxidant activities [8]. Ethnobotanically, *S. heteromalla* is used for the treatment of rheumatoid arthritis, cough with cold, stomachache, dysmenorrhea, skin diseases, cough, paralysis, women's reproductive diseases, and altitude sickness. The plant has also been found to have anti-inflammatory, cardiotonic, abortifacient, anticancer, antivenom, and antifatigue actions [9–13]. Looking at the traditional medicinal applications of *S. heteromalla*, the present study is aimed at investigating bioactive compounds involved in the suppression of oxidative stress.

2. Materials and Methods

2.1. Collection and Identification of Plant Material

A whole plant, *S. heteromalla*, was collected during March–April 2019 from the Margalla Hills National Park, Pakistan. The plant was identified with the help of the literature and submitted to the herbarium of Quaid-i-Azam University, Islamabad, Pakistan (Figure 1). The plant material was rinsed with distilled water to remove any contaminations, and then it was dried at room temperature to maintain its color and volatile compounds in it. The dried material was then pulverized to fine powder form for extraction and stored at 4 °C until a further process.

Figure 1. *Saussurea heteromalla* L.

2.2. Fractionation

The fractionation of crude extract was carried out by suspending 200 g of extract in 100 mL of water and then partitioning it with hexane, chloroform, acetone, and methanol the in order of increasing polarity by using a separating funnel. All fractions were dried using a rotary evaporator (BUCHI R-100 Rotavapor, Buchi, Essen, Germany) under high pressure and temperature (40 °C) (Figure 2).

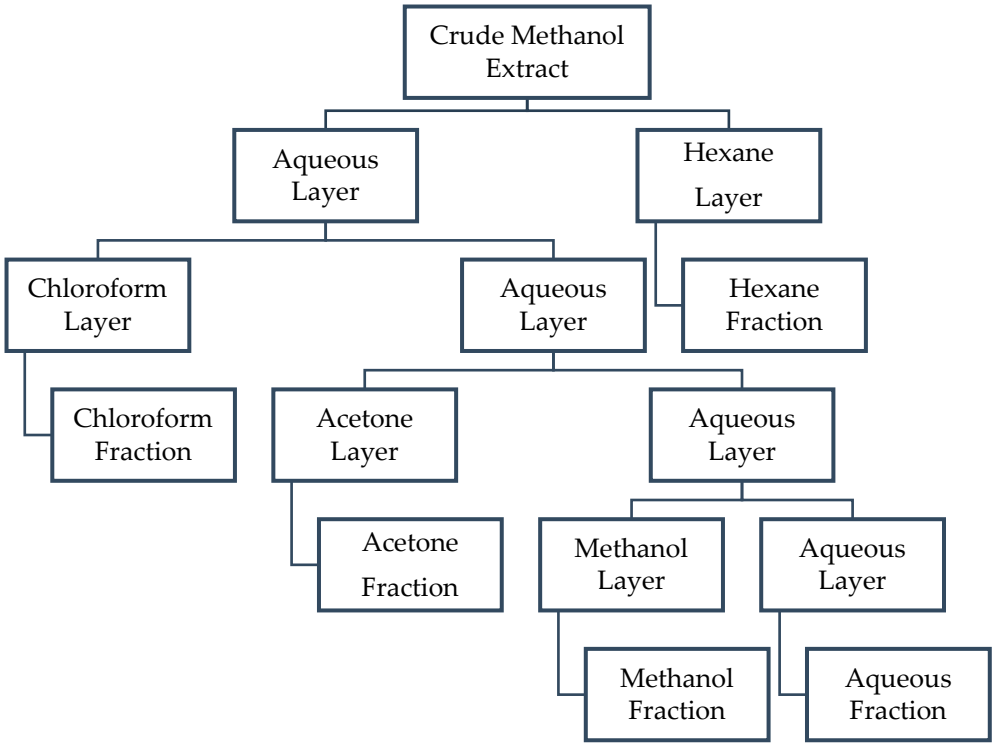

Figure 2. Summary of the scheme used for fractionation to obtain different extracts.

2.3. Free Radical Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay was carried out by following the protocol of Saboon [14]. For the experiment, a DPPH stock solution was prepared by dissolving 2.4 mg of DPPH in 100 mL of methanol. The absorbance of the stock solution was set at 0.980 ± 0.02. After that, 2 mL of the DPPH stock solution was mixed with 100 µL of the plant extract at varying concentrations (62.5–1000 µg/mL) and incubated in the dark at room temperature for 15 min. The absorbance was then measured at 517 nm. Quercetin was used as a positive control. The percentage scavenging activity was calculated with the help of the following formula:

$$\text{Percentage scavenging activity} = ((A_0 - A_1)/A_0) \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

2.4. Nitric Oxide Scavenging Assay

RAW 264.7 (mouse leukemic macrophage) cell lines were used for nitric oxide scavenging assay. A cell line was stimulated by Lipopolysaccharides (LPS) derived from *E. coli* to bring nitric oxide synthesis. The Griess colorimetric reaction method was used to carry out the assay [15]. Cells were washed and seeded in 96-well microtiter plates (2×10^5 cells/well); 100 µL of the media was added and incubated at 37 °C overnight to facilitate the attachment of cells. When the cells were fully adhered, they were treated with 10 µL of test plant extracts and incubated for 2 h. After 2 h of incubation at 37 °C with the test extracts, 50 µL of LPS (5 µg/mL) in DMEM (Dulbecco's modified Eagle medium) was

added to all the wells and again incubated for 24 h. After incubation, the supernatants from the cells were collected for nitric oxide measurement. In 96-well plates, 100 µL of cell supernatant was combined with an equal volume of the Griess reagent and kept at room temperature for 10 min; after that, absorbance was taken at 550 nm using a plate reader. Quercetin was used as a control. The percentage inhibition of nitric oxide was measured with the help of the following equation:

$$\text{Percentage inhibition of nitric oxide} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

2.5. Bioassay-Guided Purification

A dried active hexane extract of *S. heteromalla* was adsorbed on silica (Sigma-Aldrich, St. Louis, MO, USA). A sufficient quantity of silica gel was added in petroleum ether to make a slurry and then loaded to a column (size of 35 × 500 mm and pressure of 40 bar), avoiding any air bubbles. A dried sample adsorbed on silica was loaded at the top of the column. The column was then eluted, with a step gradient increasing in polarity hexane and dichloromethane, 9.5:0.5, 9:1, 8.5:0.5, 8:2, 7:3; 6:4, 5:5, 4:6, 3:7, 2:8, 1:9, and 0:10. The column was then eluted with 100% dichloromethane, then 100% ethyl acetate, followed by a mixture of ethyl acetate and methanol, 9.5:0.5, 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. Finally, the column was eluted with 100% methanol. At each step, 10 tubes of 40 mL fractions were collected, and the solvent was evaporated. The whole separation was monitored by a dual λ model 2487 absorbance detector at 280 and 254 nm (Figure S1) [16].

2.6. HPLC–DAD Analysis

High performance liquid chromatography analyses were performed on a Shimadzu LC-20AT system (Model DGU 20A3, Shimadzu, Kyoto, Japan) equipped with an LC-20AT quaternary pump. The data were acquired and processed using the LabSolutions software. The active fraction was analyzed using a reverse-phase HPLC column: Sunfire™ Prep C18 (10 mm × 250 mm, 5 µm) column. The mobile phase was composed of 4% H_2O and 96% acetonitrile (LC–MS Chromasolv®, Fluka) with 0.1% TFA (Acros Organics, Thermo Fisher Scientific, Waltham, MA, USA). Prior to HPLC injection, samples were filtered through a Chromafil® Xtra H-PTFI filter. An amount of 2 mL of sample was injected and run for 60 min at 20 °C with a flow rate of 4 mL/min [16].

2.7. GC/MS Analysis

Gas chromatography/mass spectrometry analysis of different extracts was performed by using Shimadzu QP2010 comprising an autosampler high performance gas chromatograph interfaced to a mass spectrometer with a capillary column (Rtx®-5Ms) (L. 30 mm × ID 0.25 mm × thick. 0.25 µm). The chromatographic conditions used were: initial temperature, 80 °C; final temperature, 280 °C; and injection temperature, 250 °C. The column temperature was set at 80 °C for 2 min at first, then increased to 280 °C at a heating rate of 5 °C min^{-1} and was maintained for 12 min. Helium gas (99.995%) was used as the carrier gas at a constant flow rate of 1 mL/min. GC/MS data were analyzed by using the NIST database software to check and relate with the available compounds in the database.

2.8. Statistical Analysis

All the assays were carried out in triplicate. The results were expressed as the mean of the values obtained for the replications. A statistical analysis was performed with the GraphPad Prism software (GraphPad Prism 5.0 Software Inc., San Diego, CA, USA), and IC_{50} values were calculated. Statistical significance was established at $p \leq 0.05$.

3. Results

3.1. Yield of Extraction

S. heteromalla was subjected for small-scale extraction by using different solvents (n-hexane, acetone, methanol, and water) for nitric oxide scavenging ability to find out which extract will be potent for large-scale extraction and will have high yield. During small-scale extraction, the highest yield was observed in water extracts with a percentage yield of 5.70, followed by the methanol extracts 4.30, and the lowest yield was observed in the chloroform extract with a yield percentage of 1.80 (Table 1).

Table 1. Yield percentage of *S. heteromalla* in different solvents.

Solvents	Yield (%)
Hexane	2.13
Chloroform	1.80
Acetone	2.90
Methanol	4.30
Aqueous	5.70

3.2. Free Radical Scavenging Assay

The free radical scavenging activity of *S. heteromalla* was checked with help of DPPH and nitric oxide free radicals. To check the activity, different solvent extracts (methanol, acetone, chloroform, n-hexane, and water) of the plant at varying concentrations (62.5–1000 µg/mL) were tested against free radical DPPH in triplicate. Among the different extracts, the n-hexane extract of *S. heteromalla* was found to be the most potent with a high DPPH inhibitory activity of 96.60 ± 2.31%, which was close to the control, showing 99.87 ± 1.64% inhibition at the highest concentration. The IC_{50} value of the n-hexane extract was 102.56 ± 1.48 µg/mL (Table 2). However the extraction yield of n-hexane was low as compared with the methanol and acetone extracts. In terms of activity, methanol showed 79.76 ± 0.41% with an IC_{50} value of 224.023 ± 2.67, and acetone presented 75.066 ± 0.79% with an IC_{50} value of 288.928 ± 1.58 (Table 2).

Table 2. IC_{50} values of DPPH activity.

Solvent	Concentration µg/mL					IC_{50}
	1000	500	250	125	62.5	
Methanol	79.76 ± 0.41	68.30 ± 0.82	56.57 ± 0.53	42.23 ± 0.64	31.20 ± 1.73	224.02 ± 2.67
Acetone	75.06 ± 0.79	64.23 ± 1.26	47.86 ± 0.9	32.40 ± 0.57	25.36 ± 0.73	288.92 ± 1.58
Chloroform	83.43 ± 0.48	73.98 ± 0.73	57.26 ± 0.51	51.00 ± 0.02	30.49 ± 0.26	174.88 ± 1.97
n-Hexane	96.60 ± 2.31	85.03 ± 1.72	65.93 ± 0.86	51.27 ± 2.41	32.16 ± 1.04	102.56 ± 4.48
Water	66.50 ± 1.43	53.56 ± 0.57	44.93 ± 0.94	30.90 ± 0.43	23.83 ± 0.25	434.33 ± 2.48

The values represent the averages (±SE) of three independent replicates.

3.3. Nitric Oxide Scavenging Assay

DPPH scavenging activity alone is not considered the sole test to assess the antioxidant activity of plant extracts. Therefore, nitric oxide scavenging assay is also used to check the scavenging ability of the extracts. NO is a key free radical that plays an important role in host defense response against various pathogens and regulates pathophysiological processes, such as vasodilation, neurotoxicity, and neuronal communication. Nitric oxide scavenging mitigates the propagation of inflammation as overproduction of nitric oxide causes severe toxicity, leading to cell death and tissue damage. It further results in acute or chronic inflammation. Some studies associated the overproduction of NO with cancer. The nitric oxide inhibitory activities of different solvent extracts (methanol, acetone, chloroform, n-hexane, and water) of *S. heteromalla* at varying concentrations (62.5–1000 µg/mL) were

tested against free radical DPPH in triplicate. The nitric oxide inhibitory activity of the n-hexane extract of *S. heteromalla* is found to be significant with an inhibition percentage of 87.5 ± 0.3% and an IC_{50} value of 61.67 ± 1.73 (Table 3) at the highest concentration, followed by the acetone extract, showing percentage inhibition at a high concentration of 80.9 ± 0.63%. The lowest inhibition is observed in the case of water extracts with a percentage inhibition of 67.4 ± 0.32% and an IC_{50} value of 114.84 ± 0.94 (Table 3).

Table 3. IC_{50} values of nitric oxide inhibition activity.

Solvent	Concentration µg/mL					IC_{50}
	1000	500	250	125	62.5	
Methanol	70.4 ± 0.24	65.3 ± 0.45	52.7 ± 0.19	47.5 ± 0.14	40.4 ± 0.25	187.66 ± 1.34
Acetone	80.9 ± 0.63	76.5 ± 0.12	63.9 ± 0.28	53.8 ± 0.07	37.4 ± 0.09	114.84 ± 0.94
Chloroform	80.4 ± 0.04	68.6 ± 0.01	63.5 ± 0.31	51.0 ± 0.02	44.7 ± 0.05	289.99 ±1.39
n-Hexane	87.5 ± 0.30	80.6 ± 0.23	75.5 ± 0.19	64.8 ± 0.15	50.3 ± 0.15	61.67 ± 1.73
Water	67.4 ± 0.32	63.8 ± 0.05	48.3 ± 0.13	34.9 ± 0.26	31.8 ±0.06	262.35 ± 1.28

The values represent the averages (±SE) of three independent replicates.

3.4. Bioassay-Guided Purification

n-Hexane fraction (8.75 g) was obtained with successive large-scale extraction, dried, adsorbed on silica, and run in column chromatography for isolation using different eluting solvents in view of other studies where different ratios of solvents were used for the fractionation of crude extracts to obtain active fraction [16–19]. A total of 125 different fractions were obtained, and all were subjected for nitric oxide scavenging assay. However the fractions from 20 to 45 min (fractions 6–35) showed activity (Figure 3). Active fractions from the column were subjected to thin-layer chromatography (TLC), which provided us the first source of information for the identification and separation of compounds (Figure S2) [20]. On the basis of TLC results, fraction 11 (90% hexane and 10% dichloromethane) is found to be most potent for further evaluation (Figure S3).

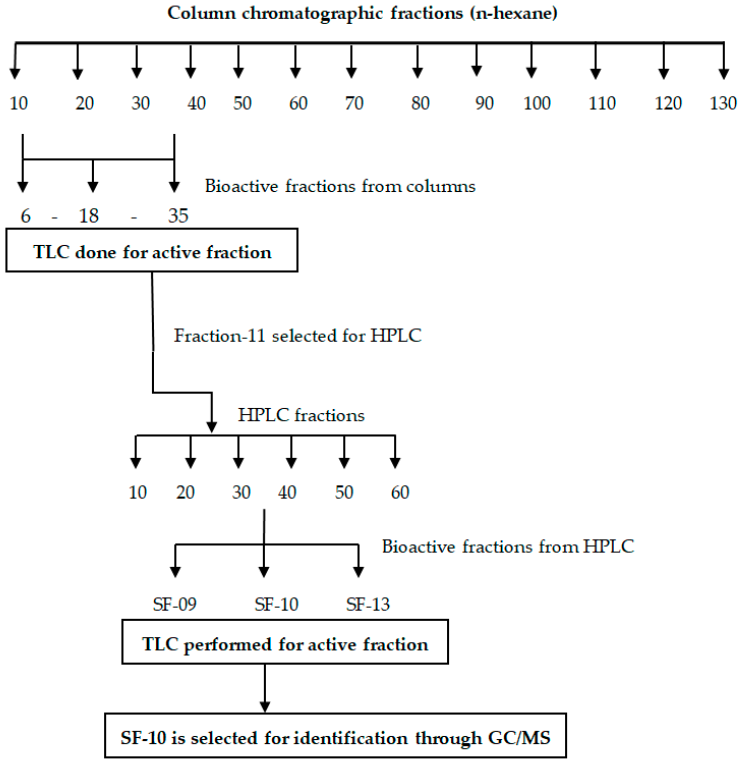

Figure 3. Summary of the identification scheme for the compound.

3.5. HPLC–DAD

In the search of a bioactive compound with free radical scavenging activity, the active fraction 11 (F-11) was further separated with the help of HPLC–DAD, which is a useful technique for the identification of phenolic, alkaloid, and steroid compounds [21,22]. This technique has been proved to be more powerful and useful for the rapid profiling and identification of active components when coupled with other chromatographic techniques. In the present study, analysis for F-11 was performed with 90% acetonitrile and 10% water, 60 different subfractions were obtained, and all these fractions were subjected to nitric oxide inhibition activity. From F-11, the subfractions SF-09, Sf-10, and SF- 13 showed activity and active peaks in an HPLC chromatograph, and TLC was used to test the purity of a subfraction (Figure 3). All the active subfractions were subjected to TLC to check the purity. On the basis of a TLC subfraction, SF-10 was selected for further analysis and collected at an amount of 2.5 mg for GC/MS analysis.

3.6. GC/MS Analysis

GC/MS is one of the reliable techniques for analyzing hundreds of chemical compounds, including important secondary metabolites, such as glycosides, phenols, organic acids, amino acids, and fatty acids. It effectively works in chemical isomers, which all have an equal mass spectrum. Besides essential oils, most of the diethyl ether extracts of medicinal plants have been reported to be analyzed by this method. In the present study, the bioactive subfraction (SF-10) was subjected to GC/MS analysis. In the mass spectra of the GC/MS graph, the compound peak was observed at 24.13 min. The mass spectra of the compound showed the highest molecular ion peak at m/z 204 (100.0%), 249 (99.9%), 112 (83.3%), 232 (80.4%), 70 (73.3%), and 41 (64.2%), and the base peak at m/z 360 (50.4%), as shown in Figure 4. On the basis of the mass spectrum, the compound was analyzed with the NIST database, and the active compound was identified as 2-ethylhexyl 2-cyano-3, 3-diphenylprop-2-enoate, commonly known as octocrylene, with two benzene rings (Figure 5); the chemical formula of the compound is $C_{24}H_{27}NO_2$, with a molecular weight of 361.3 g/mol. Physically, the compound is insoluble in water, with a density of 1.05 g/cm^3 and a melting point of 14 °C.

The identified compound, when subjected to antioxidant activity and nitric oxide inhibitory activity, proved to be active and has shown almost equal inhibition of free radical as that of a positive control (Table 4).

Table 4. Free radical scavenging activity of the identified compound.

Compounds	DPPH Scavenging Ability	NO Inhibition (%)
Octocrylene	95.31 ± 0.32	91.48 ± 0.75
Quercetin	98.17 ± 0.07	94.63 ± 0.03

The values represent the averages (±SE) of three independent replicates.

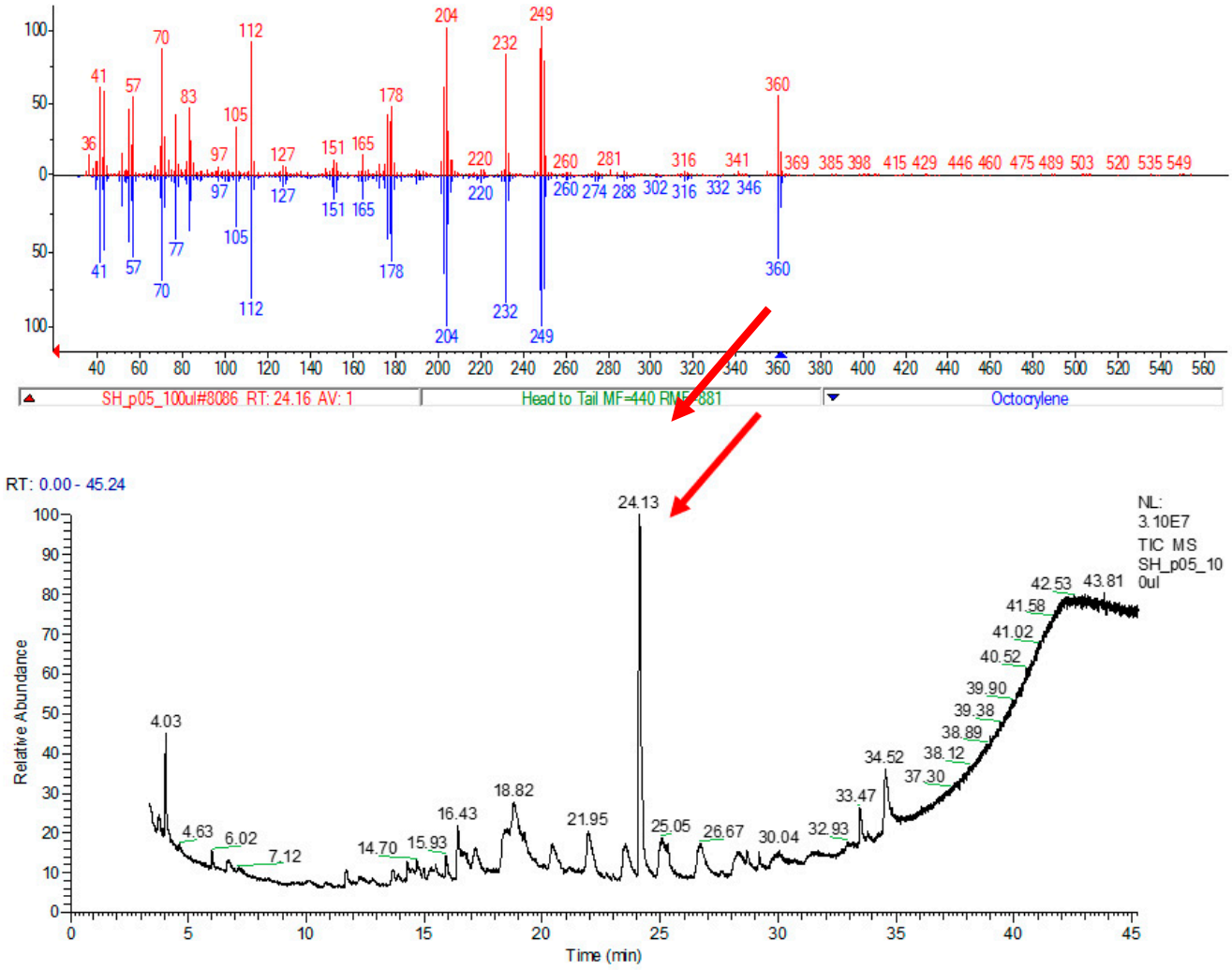

Figure 4. GC/MS absorption spectra of SF-10.

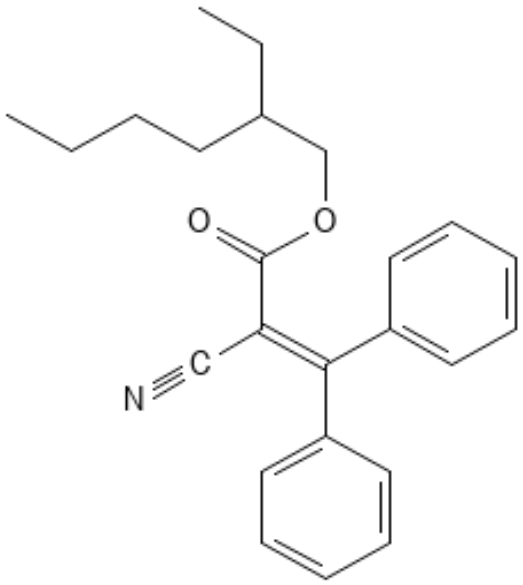

Figure 5. Identified compound and its structure.

4. Discussion

The inhibitory activity of *S. heteromalla* extracts validated its effective ethnobotanical use against tumor and inflammation, and in our case, the extracts were also proved to have good inhibitory activity against free radical. A literature study also revealed its effectiveness as an antitumor, antioxidant, and antimicrobial agent. The crude methanolic extract of *S. heteromalla* already showed its potency against different tumor cell lines and its antimicrobial activity against Gram-positive and Gram-negative bacterial and fungal strains [23]. In the present study, we used different polarity solvents (n-hexane, chloroform, acetone, methanol, and water) for our desired compound separation. In our case, the presence of our desired bioactive compound was found in nonpolar solvents as in biological activities, and the nonpolar solvent was found to be good in its bioactivity and inhibition against free radicals. The free radical scavenging activity of extracts was assessed with DPPH. DPPH is a synthetic-nitrogen-centered free radical, purple in color, and during reduction by an antioxidant, it changes into DPPHH, a yellow-color product, by accepting hydrogen or an electron from it [24]. DPPH scavenging assay is used by the researcher due to its simple, rapid, sensitive, and reproducible procedure. Therefore, DPPH scavenging assay in cell-free systems is often considered by researchers for antioxidant studies before further research on cell lines and/or animal models. Previously, DPPH assay of polar extracts (crude methanol) of *S. heteromalla* did not show enough antioxidant activity [23]. That finding is in agreement with the present study as polar extracts, methanol, and water are not as good as nonpolar extracts. This may be due to different extraction efficiencies of solvents. The phytochemicals extracted with the polar solvent of *S. heteromalla* may lack the binding sites for free radicals, such as DPPH and NO. Our results are in agreement with those of a previous work on the cytotoxic effect of *S. heteromalla*, where nonpolar extracts were observed to be good for bioassay-based identification. In another study conducted on the anti-inflammatory activity of *S. heteromalla*, ethyl acetate extract was found to be better for bioassay-guided isolation as compared with methanol [25]. The selection of an ideal solvent is crucial for the isolation and assessment of biological activities. Moreover, a pervious study on the family Asteraceae showed that the presence of a high content of medicinally important phytochemicals may be responsible for the high antioxidant activity in this study.

The active fraction was subjected to bioassay-guided isolation. Bioassay-guided isolation involves the process of separation of phytochemicals in a mixture using various analytical methods on the basis of results obtained from biological testing. The process begins with the testing of an extract bioactivity, followed by fractionation. The active fractions are then subjected to an isolate pure active compound. For the isolation of an active compound, chromatography is useful; however, it proves to be invaluable in identification when a larger number of compounds have to be examined rapidly with both high sensitivity and selectivity. Moreover, single techniques can never be trusted to analyze a plant sample, so the verification should be performed with more advanced methods, such as mass spectrometry (MS) or GC/MS. In our study, we used conventional chromatographic techniques, such as column chromatography, TLC, HPLC, and GC/MS, along with a bioassay-guided approach, for obtaining an extract, fraction, and bioactive compound from *S. heteromalla* with free radical scavenging activity. The HPLC and GC/MS techniques are more suitable techniques for bioactive drug discovery. They have been recognized as essential tools for potential drug discovery from herbal medicines due to their pronounced resolution and accuracy [26]. The mass spectrum of this technique is a powerful analytical tool for assessing structural information by evaluating the peaks, relative intensities, exact mass, molecular formula, and density of the compound [27]. Many phytochemicals have been identified through this technique from the genus *Saussurea*, such as limonene, apigenin, saussurenoside, and pathalic acid [28]. Some researchers have reported the genus *Saussurea* to be rich in phenolics due to which this family is well known for its free radical scavenging activity as many researchers correlate its antioxidant activity with the presence of phenols and flavonoids. Researchers have pointed out the presence

of quercetin and kaempferol in *S. salicifolia* [29], while the presence of ester compounds (sinkiangenorin A and sinkiangenorin B) was detected during a work conducted on seeds of *S. involucrate*; the isolated compound, when tested against a tumor cell line (A549, MCF-7, HeLa), showed high antitumor activity [30]. In recent years, researchers carried out a comprehensive analysis of the antiaging and antioxidant activity of the Asteraceae family, which resulted in the identification of some important antioxidant compounds, such as oxyphyllacinol, valine, citric acid, and sugiol from *Synedrella nodiflora* L. [31].

The identified compound of our study, 2-ethylhexyl 2-cyano-3, 3-diphenylprop-2-enoate, is an important part of the cosmetic industry, being used in cosmetic products as sunscreen and skin moisturizer [7]. 2-Ethylhexyl 2-cyano-3, 3-diphenylprop-2-enoate is one of the ultraviolet (UV) filters that are commonly added to chemical sunscreens [32]. Our study reports that this compound was observed for the first time in this family and much less reported from the botanical source previously. There is only a single report on this compound being detected in *Globularia alypum* L. (G. A.) [33]. It seems to be a potential novel compound from this plant source.

5. Conclusions

S. heteromalla was known to possess medicinal efficacy and has been used in traditional ethnobotany. Bioassay-guided compound extraction systematically analyzed the presence of a bioactive phytochemical that showed its extraction affinity to nonpolar solvents. The n-hexane extracts of *S. heteromalla* showed free radical scavenging activity due to the presence of 2-ethylhexyl 2-cyano-3, 3-diphenylprop-2-enoate. This supports the traditional medicinal use of this plant for the treatment of different ailments, including anti-inflammation and tumor and as antioxidant. Owing to the free radical scavenging activity of the compound, the plant extracts can be used as a natural source of antioxidant. The compound has been reported once from a botanical source and seems novel from this plant family. The literature study of this compound confirmed its use in cosmetics as a skin protectant against UV radiation. Therefore, further studies are suggested in order to utilize it as a natural sun protectant at the industrial level. It is also suggested that the isolated compounds should be studied against different biological activities and cell lines to identify their other potential activities and toxicity. Furthermore, in vivo and mechanistic studies are encouraged to find out possible synergistic effects with other compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/separations10020107/s1, Figure S1: Absorption spectra from column chromatography at wavelength of 254–280 nm. Red spot indicates active peaks. Figure S2: TLC separation and identification of active peaks. Figure S3: HPLC-DAD absorption spectra of farction11, area with Red Cross are active peaks.

Author Contributions: S. and Y.B.: conceptualization, investigation, and writing—original draft. Y.B., K.M., S.A.A. and S.K.: conceptualization, project administration, supervision, and writing—original draft. S.A.A. and Y.B.: Data analysis. T.A., S.G., M.H., S.A.A. and A.Q.: proofreading. A.Q. and S.A.A.: collection of review of literature and funding. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available upon fair request from the corresponding authors.

Acknowledgments: The authors would like to thanks the support of Prince Sultan University for paying the article processing charges of this publication.

Conflicts of Interest: The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Altemimi, A.; Lakhssassi, N.; Baharlouei, A.; Watson, D.G.; Lightfoot, D.A. Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts. *Plants* **2017**, *6*, 42. [CrossRef]
2. Isman, M.B. Bioinsecticides based on plant essential oils: A short overview. *Z. Naturforsch. C.* **2020**, *75*, 179–182. [CrossRef]
3. Quan, N.V.; Xuan, T.D.; Anh, L.H.; Tran, H.D. Bio-guided isolation of prospective bioactive constituents from roots of *Clausena indica* (Dalzell) Oliv. *Molecules* **2019**, *24*, 4442. [CrossRef]
4. Basri, A.M.; Taha, H.; Ahmad, N. A review on the pharmacological activities and phytochemicals of *Alpinia officinarum* (Galangal) extracts derived from bioassay-guided fractionation and isolation. *Pharmacogn. Rev.* **2017**, *11*, 43. [CrossRef] [PubMed]
5. Cefali, L.C.; Ataide, J.A.; Moriel, P.; Foglio, M.A.; Mazzola, P.G. Plant-based active photoprotectants for sunscreens. *Int. J. Cosmet. Sci.* **2016**, *38*, 346–353. [CrossRef] [PubMed]
6. Bingol, M.N.; Bursal, E. LC-MS/MS analysis of phenolic compounds and *in vitro* antioxidant potential of *Stachys lavandulifolia* Vahl. var. brachydon Boiss. *Int. Lett. Nat. Sci.* **2018**, *72*, 28–36. [CrossRef]
7. Berardesca, E.; Zuberbier, T.; Sanchez, V.M.; Marinovich, M. Review of the safety of octocrylene used as an ultraviolet filter in cosmetics. *J. Eur. Acad. Dermatol. Venereol.* **2019**, *33*, 25–33. [CrossRef] [PubMed]
8. Bursal, E.; Yılmaz, M.A.; Izol, E.; Türkan, F.; Atalar, M.N.; Murahari, M.; Ahmad, M. Enzyme inhibitory function and phytochemical profile of *Inula discoidea* using in vitro and in silico methods. *Biophys. Chem.* **2021**, *227*, 1–14. [CrossRef]
9. Ahmad, M.; Zafar, M.; Shahzadi, N.; Yaseen, G.; Murphey, T.M.; Sultana, S. Ethnobotanical importance of medicinal plants traded in herbal markets of Rawalpindi- Pakistan. *J. Herb. Med.* **2018**, *11*, 78–89. [CrossRef]
10. Amjad, M.S.; Arshad, M.; Saboor, A.; Page, S.; Chaudhari, S.K. Ethnobotanical profiling of the medicinal flora of Kotli, Azad Jammu and Kashmir, Pakistan: Empirical reflections on multinomial logit specifications. *Asian Pac. J. Trop. Med.* **2017**, *10*, 503–514. [CrossRef]
11. Ali, K.; Khan, N.; Rahman, I.; Khan, W.; Ali, M.; Uddin, N.; Nisar, M. The ethnobotanical domain of the Swat Valley, Pakistan. *J. Ethnobiol. Ethnomed.* **2018**, *14*, 1–15. [CrossRef]
12. Rehman, K.; Mashwani, Z.R.; Khan, M.A.; Ullah, Z.; Chaudhary, H.J. An ethnobotanical perspective of traditional medicinal plants from the Khattak tribe of Chonthra Karak, Pakistan. *J. Ethnopharmacol.* **2015**, *165*, 251–259. [CrossRef] [PubMed]
13. Shah, A.A.; Khan, Z.; Ramzan, M.; Saba, R. Ethnoecological studies of herbs and shrubs of Miani Sahib Graveyard, Lahore city, Punjab, Pakistan. *J. Bioresour. Manag.* **2016**, *3*, 33–34. [CrossRef]
14. Saboon; Arshad, M.; Bibi, Y.; Ahmad, M.S. Multi-elemental analysis of some traditional medicinal plants from Marghalla Hill National Park, Pakistan. *J. Pure Appl. Agric.* **2022**, *7*, 29–42.
15. Adebayo, S.A.; Ondua, M.; Shai, L.J.; Lebelo, S.L. Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants. *J. Inflamm. Res.* **2019**, *12*, 195. [CrossRef] [PubMed]
16. Kerkoub, N.; Panda, S.K.; Yang, M.R.; Lu, J.G.; Jiang, Z.H.; Nasri, H.; Luyten, W. Bioassay-guided isolation of anti-candida biofilm compounds from methanol extracts of the aerial parts of *Salvia officinalis* (Annaba, Algeria). *Front. Pharmacol.* **2018**, *9*, 1418. [CrossRef]
17. Bajpai, V.K.; Majumder, R.; Park, J.G. Isolation and purification of plant secondary metabolites using column-chromatographic technique. *Bangladesh J. Pharmacol.* **2016**, *11*, 844–848. [CrossRef]
18. Khalil, R.; Ali, Q.; Hafeez, M.; Malik, A. Phenolic acid profiling by RP-HPLC: Evaluation of antibacterial and anticancer activities of *Conocarpus erectus* plant extracts. *Biol. Clin. Sci. Res. J.* **2020**, *1*, 45–46. [CrossRef]
19. Yosri, M.; Amin, B.H.; Abed, N.N.; Elithy, A.S.; Kareem, S.M.; Sidkey, N.M. Identification of novel bioactive compound derived from *Rheum officinalis* against campylobacter jejuni NCTC11168. *Sci. World J.* **2020**, *20*, 1–9. [CrossRef]
20. Colpo, E.; Vilanova, C.D.D.; Reetz, L.G.B.; Duarte, M.M.; Farias, I.L.G.; Meinerz, D.F.; Rocha, J.B.T. Brazilian nut consumption by healthy volunteers improves inflammatory parameters. *Nutrition* **2014**, *30*, 459–465. [CrossRef]
21. Barbosa, F.V.M.; Waczuk, E.P.; Kamdem, J.P.; Abolaji, A.O.; Lacerda, S.R.; Costa, J.G.M.; Posser, T. Phytochemical constituents, antioxidant activity, cytotoxicity and osmotic fragility effects of Caju (*Anacardium microcarpum*). *Ind. Crops Prod.* **2014**, *55*, 280–288. [CrossRef]
22. Batool, A.; Miana, G.A.; Muddassir, M.; Khan, M.A.; Zafar, S. In vitro cytotoxic, antioxidant, antibacterial and antifungal activity of *Saussurea heteromalla* indigenous to Pakistan. *Pak. J. Pharm. Sci.* **2019**, *32*, 2771–2777. [CrossRef]
23. Batool, A.; Miana, G.A.; Alam, M.; Khan, M.T.; Muddassir, M.; Zaman, W.; Khan, S.; Aman, A.; Khusro, A.; Arasu, M.V.; et al. Bioassay-guided fractionation and isolation of Arctigenin from *Saussurea heteromalla* for in vitro and in silico cytotoxic activity against HeLa cells. *Physiol. Mol. Plant Pathol.* **2022**, *117*, 101749. [CrossRef]
24. Saboon; Arshad, M.; Ahmad, M.S.; Mashwani, Z.R. Fermentation Enhances Redox Protective Activities of *Gymnosporia royleana* Wall. ex Lawson Extracts. *Iran. J. Sci. Technol. Trans. A: Sci.* **2019**, *43*, 15–23. [CrossRef]
25. Saklani, A.; Hegde, B.; Mishra, P.; Singh, R.; Mendon, M.; Chakrabarty, D.; Kulkarni-Almeida, A.A. NF-κB dependent anti-inflammatory activity of chlorojanerin isolated from *Saussurea heteromalla*. *Phytomedicine* **2012**, *19*, 988–997. [CrossRef]
26. Henke, M.T.; Kelleher, N.L. Modern mass spectrometry for synthetic biology and structure-based discovery of natural products. *Nat. Prod. Rep.* **2016**, *33*, 942–950. [CrossRef]
27. Zhao, J.; Zhao, Y.; Hu, C.; Zhao, C.; Zhang, J.; Li, L.; Xu, G. Metabolic profiling with gas chromatography–mass spectrometry and capillary electrophoresis–mass spectrometry reveals the carbon–nitrogen status of tobacco leaves across different planting areas. *J. Proteome Res.* **2016**, *15*, 468–476. [CrossRef] [PubMed]

28. Zhao, T.; Li, S.J.; Zhang, Z.X.; Zhang, M.L.; Shi, Q.W.; Gu, Y.C.; Dong, M.; Kiyota, H. Chemical constituents from the genus Saussurea and their biological activities. *Heterocycl. Comm.* **2017**, *23*, 331–358. [CrossRef]
29. Chunsriimyatav, G.; Hoza, I.; Valášek, P.; Skrovanková, S.; Banzragch, D.; Tsevegsuren, N. Determination of Phenolic compounds in *Saussurea salicifolia* (L.) DC. by HPLC. *Czech J. Food Sci.* **2009**, *27*, 259–261. [CrossRef]
30. Seilgazy, M.; Li, J.; Aisa, H.A. Isolation of steroidal esters from seeds of *Saussurea involucrata*. *Chem. Nat. Compd.* **2017**, *53*, 1196–1198. [CrossRef]
31. Astuti, R.I.; Prastya, M.E.; Batubara, I.; Budiarti, E.; Ilmiyawati, A. Antiaging and antioxidant bioactivities of asteraceae plant fractions on the cellular functions of the Yeast *Schizosaccharomyces pombe*. *Adv. Pharmacol. Pharmaceut. Sci.* **2021**, *2021*, 2119634. [CrossRef] [PubMed]
32. Zhong, X.; Downs, C.A.; Li, Y.; Zhang, Z.; Li, Y.; Liu, B.; Li, Q. Comparison of toxicological effects of oxybenzone, avobenzone, octocrylene, and octinoxate sunscreen ingredients on cucumber plants (*Cucumis sativus* L. *). Sci. Total Environ.* **2020**, *714*, 136879. [CrossRef] [PubMed]
33. Asraoui, F.; Kounnoun, A.; Cadi, H.E.; Cacciola, F.; Majdoub, Y.O.E.; Alibrando, F.; Mandolfino, F.; Dugo, P.; Mondello, L.; Louajri, A. Phytochemical investigation and antioxidant activity of *Globularia alypum* L. *Molecules* **2021**, *26*, 759. [CrossRef] [PubMed]