Full Paper

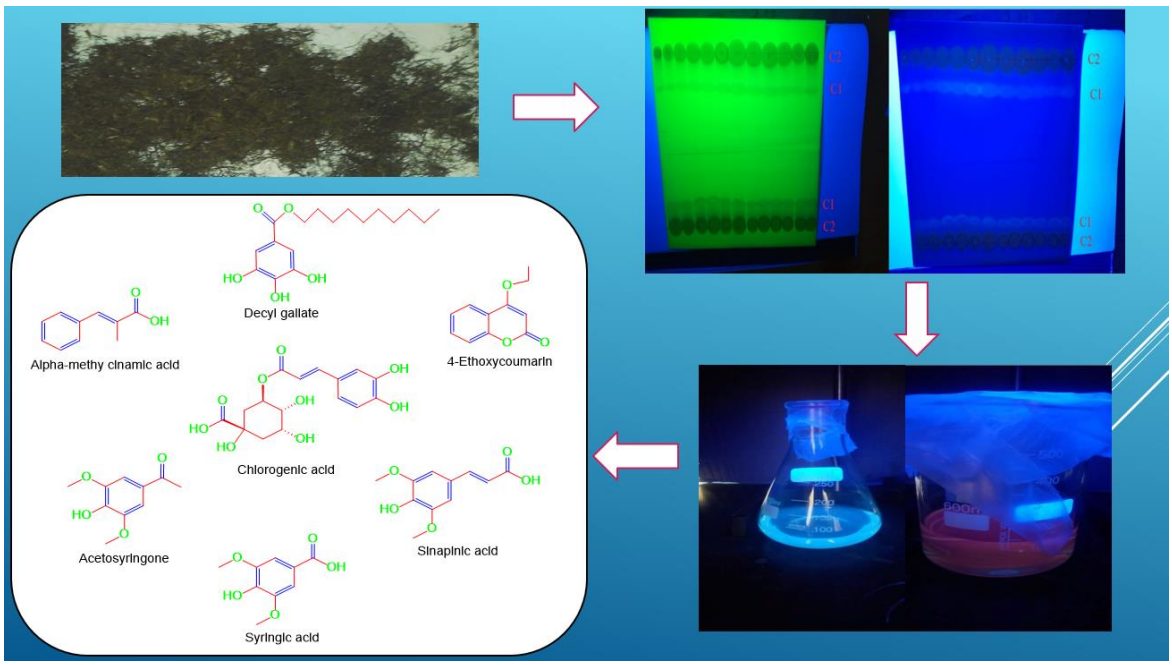
SciRad SCIENTIAE RADICES

Phytochemical analysis and the first report of decyl gallate and 4-ethoxycoumarin from marine seagrass Posidonia oceanica collected from Benghazi beach Libya

Fakhri Elabbar[(1)], Abdulsalam Alasply✉[(1)]

[(1)] Department of Chemistry, Faculty of Science, University of Benghazi, Benghazi, Libya

✉ Correspondence to: abdalslam.alasply@uob.edu.ly



Abstract: The goal of this research is to examine the chemical composition of the plant and identify the phytochemical constituents of the endemic seagrass species in the Mediterranean Sea, Posidonia oceanica, which has historically been used for a variety of purposes. After doing chemical screening, the existence of triterpenoid and phenolic proteins was discovered. Using preparative thin-layer chromatography and flash column chromatography techniques, seven phenolic compounds were isolated from the methanolic extracts of several plant parts. Namely decyl gallate, sinapinic acid, alpha-methyl cinnamic acid, acetosyringone, and chlorogenic acid from leaves, 4-ethoxycoumarin from rhizomes, and syringic acid from roots. The

structures of these compounds were elucidated using spectroscopic methods such as 'H and 13C-NMR. The spectra were analyzed and visualized using Mnova software and compared with standard spectra from the Willy spectra database, biological magnetic resonance data bank, and chemical book. This is the first report of decyl gallate and 4-ethoxycoumarin from the plant. This study contributes to the understanding of the phytochemical profile of Posidonia oceanica and provides valuable insights into its potential applications in various fields, including medicine, agriculture, and industry.

Keywords: Posidonia oceanica, Seagrass, phytochemistry, NMR, Libya

Received: 2024.04.26
Accepted: 2024.06.27
Published: 2024.06.30
DOI: 10.58332/scirad2024v3i2a05

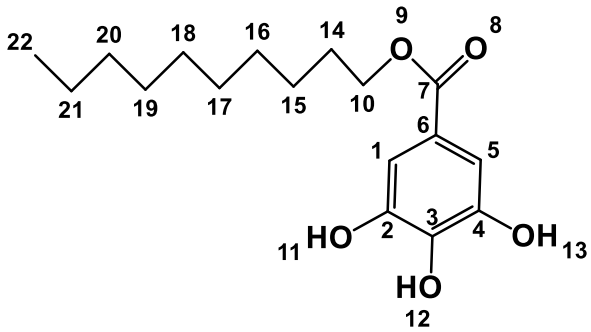
Introduction

Oceans are about 70% of the earth's plant area, a large habitat with huge diversity of diverse and unique organisms for its new and rich area of natural product research, about 30,000 compounds were reported in 2008; more than 1000 compounds were discovered every year [1]. The Mediterranean Sea has a rich biodiversity area of about 17,000 marine species, including about 27% of bacteria and archaea. 42% Animalia 14% are invertebrate and 5% are plants with this unique diversity of marine life, and 30% are endemic species [2]. Seagrass plants in the Alismatales order are the only marine angiosperm plants with more than 60 species in the four families Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae. They are distributed in 191 counters with meadows ranging from 177 000 to 600 000 km^2 [3]. There are nine species of Posidonia genus eight in Australia, and Posidonia oceanica is endemic to the Mediterranean Sea [4], As traditional medicine, it was used as medicine for sore throats and skin problems in ancient Egypt and Italy. It was also used to treat inflammation, irritation, and lower limb pain; it was even used as a remedy for acne and to treat colitis in Italy and Libya. In the sixteenth century, Posidonia oceanica was used as padding for cushions and mattresses for people with tuberculosis to prevent respiratory infections in Anatolia and even as a treatment for diabetes and hypertension [5]. In Cyrenaica, the eastern region of Libya, Posidonia oceanica leaf was used as a traditional medicine for colitis disease [6]. The leaf extract is known for antioxidant activity related to the high phenolic compound concentration in the plant [7], anti-diabetic activity [8], and antimicrobial activity [9], the hydrophilic extract of leaves can prevent HT1080 human fibrosarcoma cell line invasion [10], and the methanol extract has anti proliferative activity against the HepG2 cell line,

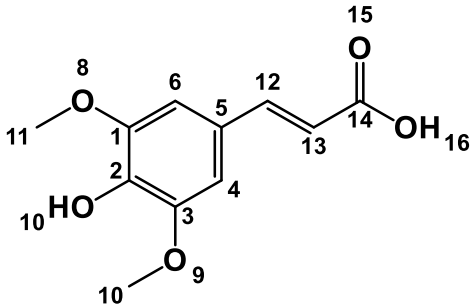
Hep2 cell line, and HCT116 cell line, the plant balls methanol extract showed activity against the HepG2 cell line, MCF7 cell line, and HCT116 cell line, the both extracts show antiviral activity against the H5N1 virus [11], the rhizome shows antifungal activity[12], the root ethanol extract has inhibition of the acetylcholinesterase enzyme butyrylcholinesterase (BChE) enzyme which is related to Alzheimer disease and inhibition of the enzyme Trypsin TYR enzyme which is related to Parkinson's disease [13] Posidonia oceanica was the center of attraction for studies from the nineties of the last century until the present day. According to [14], the first compound was reported in 1995 by [15]. From that time, more than 50 compounds were reported, including 30 compounds from phenols. It is a derivative of phloroglucinol, pyrocatechol, and pyrogallol, which are two of the tannins that form unites, phenyl methane, phenyl ethan, and phenyl propane derivatives. The larger phenolic compounds include chalcones, which were reported two times and four times for flavonoids [16]. In 2014, the lignin family was reported in the plant rhizome by pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) [17]. In 2012, the first neolignans were reported [18]. There are two reports of terpenes first in Italy. 5-α-Cholestane derivatives and cholest-5-ene derivatives The compound structures were determined by HNMR in 1984 [19]. The second report was in 2013. In Tunisia of novel methylated sesquiterpene the structure determined by 1D and 2D NMR [20]. For volta organic compounds, the 2018 study was based around the Adriatic Sea of Italy the Headspace-solid phase microextraction (HSSPME) was the extraction method, and compounds were detected with gas chromatography-mass spectrometry (GC-MS/FID). reported compounds are pentadecane, heptadecane, nonadecane, and dimethyl sulfide [21]. Our team reports sixteen compounds from hexane Soxhlet extracts of the leaves, rhizomes, and roots, with the first discovery of 3-ethyl-5-(2-ethylbutyl) octadecane, phytol, phytyl acetate, and 6,10,14-trimethylpentadecan-2-one [22]..

Experimental part

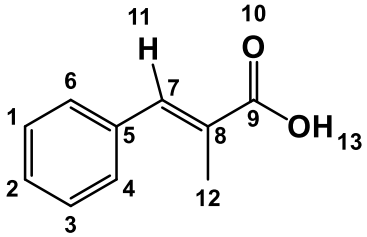
Characteristics of compounds



Decyl gallate (**1**) $C_{17}H_{26}O_5$ [23] was separated from leaves first column in two fractions first fraction L-1 in solvent system 10:90 ethyl acetate: hexane as pure blue band and as mixture with red band in the second fraction L-2 at solvent system 20:80, 30:70 ethyl acetate: hexane the compound was isolated from the mixture by preparative thin-layer chromatography and was registered as sample number 3 in NMR analysis, in ^{1}H NMR (DMSOd6, 500 MHz), in ^{1}H NMR (DMSO-d^6, 500 MHz), δ 0.85 (3H, t, J = 7.2 Hz, H-22), 1.27 (12H, m, H-16-21), 1.36 (2H, ddd, J= 8.58, 5.96,2.86 Hz, H-15), 1.63 (2H, dt, J = 8.34, 6.96 H-14), 4.14 (2H, t, J = 6.52 Hz, H-10), 6.93 (2H,s, 1H), 8.92 (1H, s, H-12), 9.24 (2H, s, H- 12,11), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 13.89 (C-22), 22.03 (C-21), 25.50 (C-15), 28.26 (C-19,18,17,16), 28.59 (C-14), 31.89 (C-20), 63.92 (C-20), 108.46 (C-20), 119.59 (C-20), 138.32 (C-20), 145.53 (C-20), 165.84 (C-20).

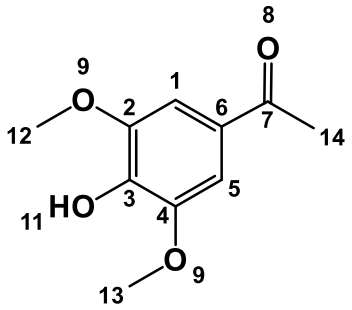


Sinapinic acid (**2**) $C_{11}H_{12}O_5$ [24,25] was isolated from preparative TLC of leaves L-3 fraction with solvent system 10:90 ethyl acetate: hexane and was registered as sample number 2 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz), δ 3.80 (6H, s, H-11,10), 6.42 (1H, d, J= 15.89 Hz, H-13), 6.98 (2H, s, H- 6,4), 7.50 (1H, d, J= 15.87 Hz, H-12), 8.91 (1H, s, H-7), 12.14 (1H, s, H-16), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 56.26 (C11,10), 106.84 (C-6,4), 115.94 (C-13), 124.26 (C-5), 138.09 (C-2), 144.74 (C-12) 148.36 (C-3,1), 167.80 (C-14).

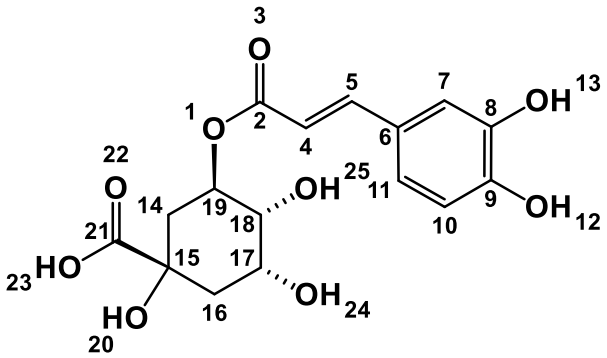


α-Methyl cinamic acid (**3**) $C_{10}H_{10}O_2$ [26,27] was isolated from preparative TLC of leaves L-4 fraction with solvent system 25:75 methanol: chloroform and was registered as sample number 1 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz), δ 2.02 (3H, d, J = 1.54 Hz, H-12), 7.35 (1H, t, J= 7.04 Hz, H-2), 7.42 (2H, t, J= 7.59 Hz, H- 3,1), 7.46 (2H, d, J = 6.97 Hz, H-6,4), 7.60 (1H, s, H-11), 12.52 (1H, s, H-13), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 14

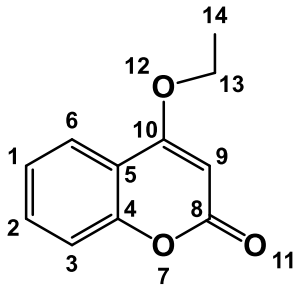
(C-12), 128.52 (C-2), 128.64 (C-3,1), 128.89 (C-6,4), 129.72 (C-5), 135.70 (C-8), 137.85 (C-7), 169.68 (C-9).



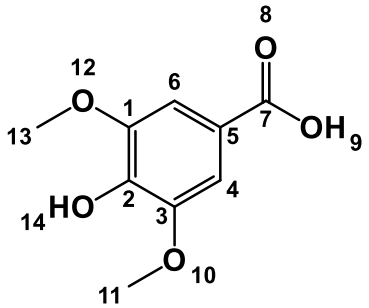
Acetosyringone (**4**) $C_{10}H_{12}O_4$ [28,29] was isolated from preparative TLC of leaves L-5 fraction with solvent system 10:90 diethyl ether: hexane and was registered as sample number 6 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz δ 2.52 (3H, s, H-14), 3.83 (6H, s, H-13,12), 7.23 (2H, s, H-5,1), 9.34 (1H, s, H-11), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 26.66 (C-14), 56.68 (C-13,12), 106.13 (C-5,1), 127.99 (C-6), 141.24 (C-3), 147.87 (C-4,2), 196.33 (C-7).



Chlorogenic acid (**5**) $C_{16}H_{18}O_9$ [30,31] was isolated from preparative TLC of leaves L-6 fraction with solvent system 10:90 methanol: chloroform and was registered as sample number 8 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz), δ 1.78 (1H, dd, J = 13.02,7.76 Hz, H-16), 1.94 (1H, dd, J = 13.27, 3.52 Hz, H-14), 2.02 (2H, dt, J= 13.35,5.14 Hz, H-16,14), 3.57 (1H, s, H-18), 3.93 (1H, s, H-17), 4.78 (1H, s, H-25), 4.93 (1H, s, H- 24), 5.07 (1H, dt, J = 11.01, 5.5 Hz, H- 19), 5.55 (1H, s, H- 20), 6.15 (1H, d, J = 15.9 Hz, H- 4), 6.77 (1H, d, J = 8.16 Hz, H- 10), 6.98 (1H, dd, J = 8.2,2 Hz, H- 11), 7.04 (1H, d, J = 2.05 Hz, H- 7), 7.42 (1H, d, J = 15.9 Hz, H- 5), 9.16 (1H, s, H- 12), 6.90 (1H, s, H- 13), 12.51 (1H, s, H- 23 in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 36.33 (C-14), 37.22 (C-16), 68.33 (C-17), 70.44 (C-19), 70.88 (C-18), 73.52 (C-15), 114.26 (C-7), 114.88 (C-4), 115.77 (C-10), 121.37 (C-11), 125.77 (C-6), 144.99 (C-5), 145.77 (C-8), 148.35 (C-9), 166.02 (C-2), 175.57 (C-21).



4-Ethoxycoumarin (**6**) $C_{11}H_{10}O_3$ [32,33] was isolated from preparative TLC of rhizomes RH-1 fraction with solvent system 10:90 ethyl acetate: hexane and was registered as sample number 5 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz),), δ 1.43 (3H, t, J = 6.98 Hz, H-14), 4.25 (2H, q, J = 7.03 Hz, H-13), 5.85 (1H, s, H-9), 7.34 (1H, ddd, J = 8.27,7.32,1.11 Hz, H-1), 7.37 (1H, dd, J = 8.31,1.12 Hz, H-3), 7.64 (1H, ddd, J = 8.94,7.27.1.67 Hz, H-2), 7.77 (1H,dd, J = 7.93,1.62 Hz, H-6), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 13.85 (C-14), 65.39 (C-13), 90.37 (C-9), 115.16 (C-5), 116.49 (C-3), 122.72 (C-1), 124.04 (C-6), 132.60 (C-2), 152.72 (C-4), 161.63 (C-10), 164.80 (C-8).



Syringic acid (**7**) $C_9H_{10}O_5$ [34] was isolated from preparative TLC of roots T-1 fraction with solvent system 10:90 ethyl acetate: hexane and was registered as sample number 4 in NMR analysis, in ^{1}H NMR (DMSO-d^6, 500 MHz), δ 3.80 (6H, s, H-13,11), 7.20 (2H, s, H-6,4), 9.19 (1H, s, H- 14), 12.60 (1H, s, H-9), in ^{13}C NMR (DMSO-d^6, 126 MHz): δ 56.37 (C-13,11), 107.27 (C-6,4), 120.53 (C-5), 140.89 (C-2), 148.03 (C-3,1), 167.39 (C-7).

Material and methods

Collection of plant material

The plant was collected in September 2019 from Garyounis Beach in Benghazi City, east of Libya, and identified in the Botany Department by specialists. The plant was washed with water, and then the plant parts, including leaves, rhizomes, and roots, were separated from each other and washed with water twice to remove any remaining salts. Then, the

plant parts were dried in a dark room using an air stream from a fan at 25oC for eight days.

Apparatus

Glass beakers, soxhlet extractors, glass condensers, glass funnels, separatory funnels, glass columns, filter papers, UV analysis lamp 254, 365 nm, TLC plate holders, preparative TLC plates, and TLC silica gel GF254 plate.

Chemicals

Methanol, hexane, chloroform, ethyl actate, diethayl ethers, Distilled water, hydrochloric acid, sulfuric acid, ammonia solution, sodium chloride, sodium sulfate anhydrous, tannic acid, bismuth nitrate, potassium iodide, mercury chloride, potassium dichromate, and lead acetate.

Prepared reagents

Tannic acid solution, dragendorffs reagent, mayer's reagent, potassium dichromate solution.

Preparation of plant extracts

Maceration A

Based on [35] 1.5 kg of leaves, 1 kg of rhizomes, and 550 kg of roots were socked in methanol for 45 days. The extracts were filtered and transferred to beakers. The leaf extract volume was 7 L for the detection of any lipids that could be separated by freezing. The leaf extract was stood in an ice bath for two days, the rhizome extract was 5 L, and the volume was reduced by an air fan to 1 L and stored in the freezer for 2 days. The root extract was 5 L, and the volume was reduced by an air fan to 450 ml. The ice path and freezer were used, and there was no signal of lipid aggregation.

Maceration B

200 g of each plant part were extracted with a 2.5 L methanol-water mixture in a ratio of 80:20 for three weeks. After extraction was completed, the extracts were filtrated and transferred to beakers, and the extract volumes were reduced by an air fan to 500 ml.

Phytochemical Screening

A maceration extraction for 15 mg of each of the plant parts, leaves, rhizomes, and roots, was done in three dark bottles with 250 ml of methanol for 14 days. The bottles were shacked every two days, and the bottle cap was opened for five minutes to get any gas bubbles out. The extracts were tested as agents in a screening test based on [36, 37, 38, 39, 40, 41].

Alkaloids Screening test

a. Tannic acid test: a few drops of 10% tannic acid were added to the extract solution if with precipitate is formed it's a positive result for alkaloids and proteins.

b. Dragendorff's test: Dragendorff's reagent preparation 0.5 g of bismuth nitrate was transferred into an empty beaker, and 10 ml of concentrated hydrochloric acid was added in another beaker. 4 g of potassium iodide was added and completely dissolved in 10 ml of distilled water, and every 1 ml of the reagent was added to 2 ml of the extract in the test tube. A positive result was observed in the formation of an orange-red precipitate.

c. Mayer's test: Mayer's reagent preparation: solution A of 1.358 g of mercury chloride was dissolved in 60 ml of distilled water, solution B of 5 g of potassium iodide was dissolved in 10 ml of water, then solution A was mixed with B and distilled water was added to make the volume 100 ml. After a few drops, a positive result was observed by yellowish or white precipitate formation.

Flavonoids and tannins screening test

a. Potassium dichromate test: a few drops of 20% potassium dichromate were added to the extracts in the test tube. The positive result observed by yellow color formation indicates the presence of tannins and phenolic compounds.

b. Lead acetate test: a few drops of 10% lead acetate were added to the extract solution; if a white precipitate appeared, this indicated the presence of tannins, and if the solution color turned yellow, that proved the presence of flavonoid.

Triterpenes screening test

Saikowski test: 2 ml of extract was mixed with 2 ml of chloroform, and 3 ml and a few drops of concentrated H2SO4 were added carefully and shaken well. The positive result for the presence of terpenoids was observed by the appearance of a reddish brown color in the interphase.

TLC screening

The screening tests were carried out on a collated silica gel GF254 plate (10 x 5 cm). Each plate was divided into two areas, and two solvent systems were selected. The silica gel plates were visualized under ultra-violate light (254 and 360 nm) and exposed to ammonia viper based on [42].

Liquid-liquid extraction fractionation

Based on [43] the extracts from maceration A were separated into nonpolar, semi-polar, high-polar, and residual fractions by the liquid-liquid extraction technique at a ratio of 50:40 for the methanolic extract and three solvents: hexane, chloroform, and ethyl acetate. The process was repeated three times for each solvent, and water and sodium dichloride were used to increase the rate of extraction and separation with the chloroform

and ethyl acetate fractions. In the case of leaves and rhizomes, in the leaf fraction process, two layers of molecule aggregation were separated: the first between the methanolic layer and chloroform layer, and the second between the methanolic layer and ethyl acetate layer. These two layers and all fractions were dried and weighted. For maceration B, the extracts were separated by liquid fractionation extraction with hexane and ethyl acetate.

Fractions Thin-layer chromatography profile

All crudes from the maceration process were screened in TLC by the solvent system ethyl acetate: hexane in four ratios: 0:100, 10:90, 25:75, 50:50, and 25:75.

Flash Column chromatography

The column was prepared based on [44]. The work focused on nonpolar and semi-polar fractions a silica gel with mish 70 to 230 were used to make sure the column achieved the most possible separation ability a hand pump used to remove any air bubbles and fix any crack in the silica gel stationary phase, a uv light lamp was used for observation of the eluates Movement from the column and their Fluorescence color, and each fraction was spotted in collated TLC silica gel GF254 plate (10X5 cm) and run by different solvent systems (diethyl ether: hexane, ethyl acetate: hexane, methanol: ethyl acetate, methanol: chloroform).

Leaves column 1

The hexane crude of 0.777 g and chloroform crude of 0.444 g from the liquid-liquid fractionation of Maceration A were mixed together, and the total crude weight of 1.22 g for the first column to run by was two solvent systems ethyl acetate: hexane and methanol: ethyl acetate.

Leaves column2

The work was based on fractions from number 4 to 10 in column 1 with crude weight of 0.750 g and the column solvent system was diethyl ether: hexane.

Leaves column 3

The work was based on the residual leaves crude from the liquid-liquid extraction fractionation of Maceration B. The crude weight was 1.2 g, and three solvent systems were used: hexane, chloroform, and methanol.

Rhizomes column

The hexane crude 1.8 g and chloroform crude 138 mg from the liquid-liquid extraction fractionation of rhizomes extract from Maceration A were mixed together, the column crud was 1.938 g, the solvent systems were ethyl acetate: hexane, fraction 1,2 was mixed and moved to preparative TLC for purification, and methanol was added to the column by a 5% ratio, and two new fractions were isolated in 80% methanol and 100%

methanol. After the column was 100 methanol, a new solvent, chloroform, was added to the column in a 5% ratio, and the new fraction was separated in a 90:10 chloroform: methanol system.

Roots column

The hexane crude weight was 1.840 g for the column, and 1.540 g was used with the ethyl acetate: hexane solvent system.

Preparative thin layer chromatography

Merck DC-Fertigplatten glass TLC plates with silica gel (10x20 cm, 20x20 cm, 60F-254) were used, and ethyl acetate and hexane were the solvent systems. After the compounds separated in silica gel, the silica was cut and filtrated with a glass funnel and filtration paper to remove the compounds from the slice gel. The isolated compounds were spotted on TLC and visualized under ultra-violate light (254 and 360 nm).

Results and discussion

Flash columns result

Table 1. Leaves column 1 fraction movement in TLC by their separation system

TLC solvent system: AcOEt : Hexane	Fraction number	Visible in UV 365nm
0:100	0	No spot
10:90	1	Blue band
20:80	2	Blue and red bands
30:70	3	Blue and red bands
40:60	4	Red band and Tail
50:50	5	Red band and Tail
60:40	6	Red band and Tail
70:30	7	Red band and Tail
80:20	8	Red band and Tail
90:10	9	Red band and Tail
100:0	10	Red band and Tail
Methanol: Ethyl acetate		
0:100	0	No spot
10:90	0	No spot
20:80	1	Blue band
30:70	3	No spot
40:60	4	No spot
50:50	5	No spot
60:40	6	No spot
70:30	7	No spot
80:20	8	Blue band
90:10	9	No spot
100:0	10	No spot

For leaves, the first column fraction L-1 was isolated as a blue band in a solvent system ratio of 10:90 ethyl acetate: hexane, and L-2 was separated as a mixture of two bands, blue and red, with solvent system ratios of 20:80 and 30:70 ethyl acetate: hexane. This fraction was selected for preparative TLC purification.

Table 2. Leaves column 2 fraction movement in TLC by their separation system.

TLC solvent system Et_2O : Hexane	Fraction number	Visible in UV 365nm
0:100	0	No spot
10:90	1	No spot
20:80	2	Blue Spot and Multiple Bands
30:70	3	Blue Spot and Multiple Bands
40:60	4	Multiple bands
50:50	5	Tail
60:40	6	Tail
70:30	7	Tail
80:20	8	Tail
90:10	9	Tail
100:0	10	Tail

Table 3. Leaves column 3 fraction movement in TLC by their separation system

TLC solvent system: AcOEt : Hexane	Fraction number	Visible in UV 365nm
0:100	0	No spot
10:90	1	No spot
20:80	2	No spot
30:70	3	No spot
40:60	4	No spot
50:50	5	No spot
60:40	6	No spot
70:30	7	No spot
80:20	8	No spot
90:10	9	No spot
100:0	10	No spot
Methanol: Chloroform		
0:100	0	Blue Spot and Multiple Bands
10:90	1	Blue Spot and Multiple Bands
20:80	2	Blue Spot and Multiple Bands
30:70	3	Tail
40:60	4	Tail
50:50	5	Tail
60:40	6	Tail
70:30	7	Tail
80:20	8	Tail
90:10	9	Tail
100:0	10	Blue Spot and Multiple Bands

After the solvent system reattached 100% ethyl acetate, the methanol was added by a ratio of 5% each time to run the column, and two new fractions L-3 and L-4 were separated in solvent system ratios of 40:60, 80:20 methanol: ethyl acetate, the remand

crude fractions from 4 to 10 were mixed and dried, then investigated by column 2, and the L-5 fraction with solvent systems 20:80, 30:70 diethyl ether, and hexane was separated. The L-6 fraction was separated from the leaf residual fractions from the liquid-liquid fractionation of Maceration B in solvent systems 10:90, 20:80 methanol, and chloroform. Only the RH-1 fraction was isolated from rhizomes as blue bands in solvent system ratios of 10:90 and 20:80 of ethyl acetate: hexane, and fraction T-1 was separated from roots as blue bands in solvent system ratios of 10:90 of ethyl acetate:hexane.

Table 4. Rhizomes column1 fraction movement in TLC by their separation system

TLC solvent system: AcOEt : Hexane	Fraction number	Visible in UV 365nm
0:100	0	No spot
10:90	1	Blue band
20:80	2	Blue band
30:70	3	Tail
40:60	4	Tail
50:50	5	Tail
60:40	6	Tail
70:30	7	Tail
80:20	8	Tail
90:10	9	Tail
100:0	10	Tail

Table 5. Roots column fraction movement in TLC by their separation system.

TLC solvent system: AcOEt : Hexane	Fraction number	Visible in UV 365nm
0:100	0	No spot
10:90	1	Blue band
20:80	2	No spot
30:70	3	No spot
40:60	4	No spot
50:50	5	No spot
60:40	6	No spot
70:30	7	No spot
80:20	8	No spot
90:10	9	No spot
100:0	10	No spot

Preparative TLC result

The leaves fraction L-2 splatted to eluent C1 the blue spot which is less polar moved up in the TLC and a mixture of red bands that remained without movement by solvent system of ethyl acetate: hexane with ratio of 10:90 and with RF 0.35, eluent C1 was compared to fraction L-1 in TLC and the spot movements indicated they are the same, in addition four eluents that papers as blue spots were isolated two eluents C2 and C3 were isolated from fractions L-3 and L-4 as by solvent systems of methanol: chloroform with ratio of 20:80 and25:75 with RF 0.90 and 0.20, C5 isolated from L-5 fraction from

leaves second column with RF 0.24 by solvent systems of diethyl ether: hexane with ratio of 10:90, and from leaves third column C6 eluent was isolated from fraction L-6 with RF 0.58 by solvent systems of methanol and chloroform with a ratio of 10:90.

Table 6. Selected fractions from plant parts columns for preparative TLC.

Fraction source	Column number	Fraction symbol	Preparative TLC solvent system	Ratio	Fraction splatting
Leaves	1	L-1	AcOEt : Hexane	10:90	0
Leaves	1	L-2	AcOEt : Hexane	10:90	2
Leaves	1	L-3	MeOH : $CHCl_3$	20:80	2
Leaves	1	L-4	MeOH : $CHCl_3$ Et_2O	25:75	2
Leaves	2	L-5	Hexane	10:90	2
Leaves	3	L-6	MeOH : $CHCl_3$	10:90	2
Rhizomes	1	RH-1	AcOEt : Hexane	10:90	3
Roots	1	T-1	AcOEt : Hexane	10:90	3

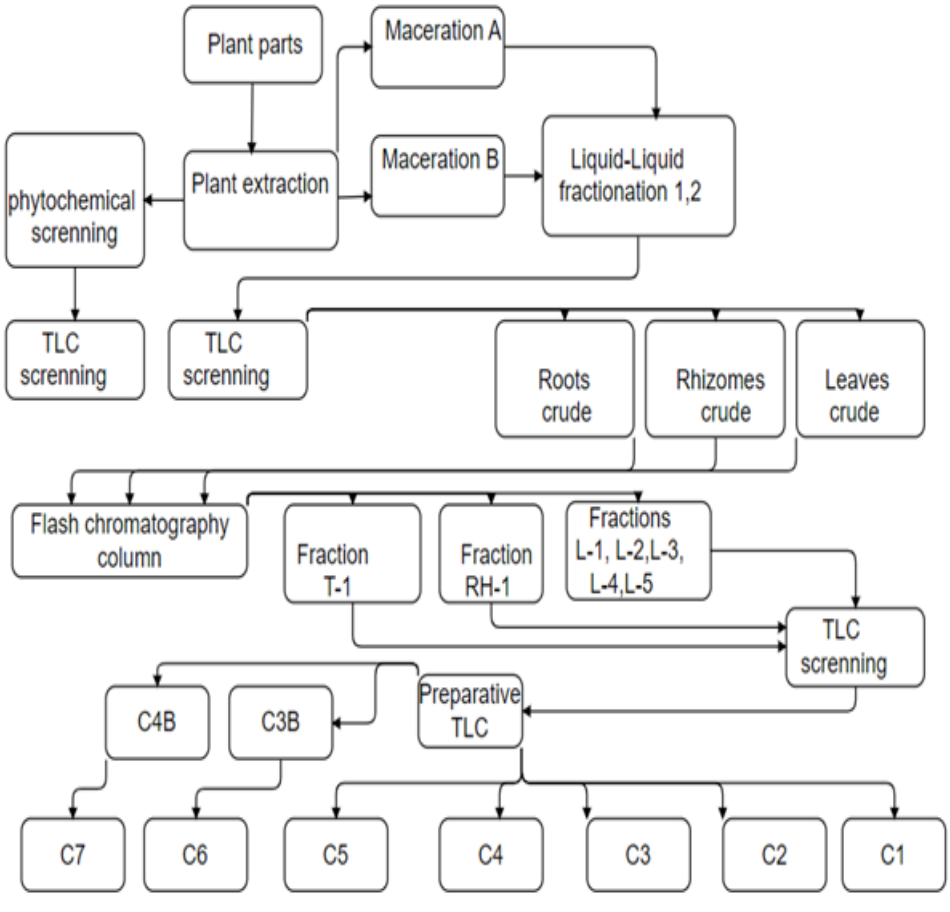


Fig 8. The process of Phytochemical analysis

For rhizomes, the RH-1 fraction was splatted into three components: C3A and C3C, two groups of bands, and C3B eluent as a blue spot, which have been purified in the second preparative TLC by a solvent system of ethyl acetate: hexane with a ratio of 10:90 and with RF 0.23 and registered as C6 eluent. The last eluent is C7 as a blue spot from roots T-

1 fraction by a solvent system of ethyl acetate: hexane with a ratio of 10:90 and with RF 0.18.

Table 7. Isolated compounds from Preparative TLC.

Fraction symbol	Preparative TLC solvent system	Ratio	Isolated eluents	RF	Weight [mg]
L-2	AcOEt : Hexane	10:90	C1	0.35	0.20
L-3	MeOH : $CHCl_3$	20:80	C2	0.90	0.18
L-4	MeOH : $CHCl_3$	25:75	C3	0.20	0.17
L-5	Et_2O : Hexane	10:90	C4	0.24	0.18
L-6	MeOH : $CHCl_3$	10:90	C5	0.58	0.21
RH-1	AcOEt : Hexane	10:90	C6	0.23	0.23
T-1	AcOEt : Hexane	10:90	C7	0.45	0.18

Conclusion

The examination of Posidonia oceanica plant components as a possible source of natural products has produced encouraging findings. It was discovered that the main substances in the plant parts extraction and chromatography separation processes were derivatives of phenolic acid. Five isolated compounds were obtained from the leaves, which turned out to be a rich source of compounds. These compounds included two derivatives of gallic acid (decyl gallate and acetosyringone) and three derivatives of cinnamic acid (sinpinic acid, alpha-methyl cinnamic acid, and chlorogenic acid). In addition, 4-ethoxycoumarin was isolated from the rhizomes, and another derivative of cinnamic acid, syringic acid, was isolated from the roots. As chemosystematics, all The isolated compounds are from the phenylpropanol pathway [45]. Among these seven phenolic compounds,.This is the first report of decyl gallate, a 4-ethoxycoumarin, from the plant. Decyl gallate is its gallic acid derivative and has been reported from Coryphantha macromeris [46]. 4-ethoxycoumarin is its coumarin derivative and has been reported from Zanthoxylum zanthoxyloides (Lam.) B. Zepernich and Timler [47], Citrus hystrix DC [48]. As a biological activity, decyl gallate has anti-fungal activity against Candida spp., Cryptococcus spp., Paracoccidioides spp., and Histoplasma capsulatum [49], anti-Paracoccidioides activity [50], 4-ethoxycoumarin is known for its anti-microbial properties [51], for the remand compounds biological activity, Sinpinic acid has anti-oxidant, anti-microbial, anti-inflammatory, anti-cancer, and anti-anxiety activity [52], chlorogenic acid has anti-oxidant and anti-spasmodic activity's, inhibition of the HIV-1 integrase, and the mutagenicity of carcinogenic compounds [53], and syringic acid has anti-oxidant, anti-microbial, and anti-inflammatory activities and is used to prevent diabetes, CVDs, cancer, and cerebral ischemia [54]. By comparing the extraction and isolation process results with

the plant parts TLC screening, column fraction, and preparative TLC, we conclude that Posidonia oceanica has a high concentration of highly polar compounds; furthermore, these compounds are found in greater amounts in the rhizomes and roots than in the leaves. Combine this with the diverse range of biological activity reported from the plant extract, this increased the possibility of separating more compounds. These results show that Posidonia oceanica is a promising natural product source. Novel bioactive compounds with a range of pharmaceutical or agricultural applications may be discovered through additional research and analysis of its chemical makeup and possible uses.

References

[1] Torres, J. P.; Schmidt, E. W. The Biosynthetic Diversity of the Animal World. *J. Biol. Chem.* **2019**, 294(46), 17684–17692. DOI: 10.1074/jbc.REV119.006130.

[2] Coll, M.; Piroddi, C.; Steenbeek, J.; Kaschner, K.; Lasram, F. B. R.; Aguzzi, J.; Ballesteros, E.; Bianchi, C. N.; Corbera, J.; Dailianis, T.; Danovaro, R.; Estrada, M.; Froglia, C.; Galil, B. S.; Gasol, J. M.; Gertwage, R.; Gil, J.; Guilhaumon, F.; Kesner-Reyes, K.; Kitsos, M. S.; Koukouras, A.; Lampadariou, N.; Laxamana, E.; de la Cuadra, C. M. L. F.; Lotze, H. K.; Martin, D.; Mouillot, D.; Oro, D.; Raicevich, S.; Rius-Barile, J.; Saiz-Salinas, J. I.; Vicente, C. S.; Somot, S.; Templado, J.; Turon, X.; Vafidis, D.; Villanueva, R.; Voultsiadou, E. The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. *PLoS One* **2010**, 5(8). DOI: 10.1371/journal.pone.0011842.

[3] McKenzie, L. J.; Nordlund, L. M.; Jones, B. L.; Cullen-Unsworth, L. C.; Roelfsema, C.; Unsworth, R. K. F. The Global Distribution of Seagrass Meadows. *Environ. Res. Lett.* **2020**, 15(7). DOI: 10.1088/1748-9326/ab7d06.

[4] Remy, F. Characterization, Dynamics and Trophic Ecology of Macrofauna Associated to Seagrass Macrophytodetritus Accumulations (Calvi Bay, Mediterranean Sea). **2016**, PhD thesis, DOI: 10.13140/RG.2.1.4935.5128.

[5] Vasarri, M.; De Biasi, A. M.; Barletta, E.; Pretti, C.; Degl'innocenti, D. An Overview of New Insights into the Benefits of the Seagrass Posidonia Oceanica for Human Health. *Mar. Drugs* **2021**, 19(9). DOI: 10.3390/md19090476.

[6] El-Darier, S. M.; El-Mokasabi, F. M. Floristic Composition and Traditional Uses of Plant Species at Wadi Alkuf, Al-Jabal Al-Akhder, Libya. *J. Agric. Environ. Sci.* **2014**, 14(8), 685–697. DOI: 10.5829/idosi.aejaes.2014.14.08.12375.

[7] Castellano, G.; Tena, J.; Torrens, F. Classification of Phenolic Compounds by Chemical Structural Indicators and Its Relation to Antioxidant Properties of Posidonia Oceanica (L.) *Delile. Match* **2012**, 67(1), 231–250.

[8] Micheli, L.; Vasarri, M.; Barletta, E.; Lucarini, E.; Ghelardini, C.; Degl'Innocenti, D.; Di Cesare Mannelli, L. Efficacy of Posidonia Oceanica Extract against Inflammatory Pain: In Vivo Studies in Mice. *Mar. Drugs* **2021,** 19(2). DOI: 10.3390/md19020048.

[9] Nasab, S. B.; Homaei, A.; Pletschke, B. I.; Salinas-Salazar, C.; Castillo-Zacarias, C.; Parra-Saldívar, R. Marine Resources Effective in Controlling and Treating Diabetes and Its Associated Complications. *Process Biochem*. **2020**, 92, 313–342. DOI: 10.1016/j.procbio.2020.01.024.

[10] Piva, G.; Fracassetti, D.; Tirelli, A.; Mascheroni, E.; Musatti, A.; Inglese, P.; Piergiovanni, L.; Rollini, M. Evaluation of the Antioxidant/Antimicrobial Performance of Posidonia Oceanica in Comparison with Three Commercial Natural Extracts and as a Treatment on Fresh-Cut Peaches (Prunus Persica Batsch). *Postharvest Biol. Technol.* **2017**, 124, 54–61. DOI: 10.1016/j.postharvbio.2016.10.001.

[11] Barletta, E.; Ramazzotti, M.; Fratianni, F.; Pessani, D.; Degl'Innocenti, D. Hydrophilic Extract from Posidonia Oceanic Inhibits Activity and Expression of Gelatinases and Prevents HT1080 Human Fibrosarcoma Cell Line Invasion. *Cell Adhes. Migr.* **2015**, 9(6), 422–431. DOI: 10.1080/19336918.2015.1008330.

[12] Far, M. M.; Marzouk, M. M.; Hussein, S. R.; Elkhateeb, A.; Abdel-Hameed, E. S. Comparative Study of Posidonia Oceanica L.: LC/ESI/MS Analysis, Cytotoxic Activity and Chemosystematic Significance. *J. Mater. Environ. Sci.* **2018**, 9(6), 1676–1682.

[13] Bernard, P.; Pesando, D. Antibacterial and Antifungal Activity of Extracts from the Rhizomes of the Mediterranean Seagrass Posidonia Oceanica (L.) *Delile. Bot. Mar.* **1989**, 32(2), 85–88 DOI: 10.1515/botm.1989.32.2.85.

[14] Orhan, I. E.; Senol, F. S.; Haznedaroglu, M. Z.; Koyu, H.; Erdem, S. A.; Yılmaz, G.; Cicek, M.; Yaprak, A. E.; Ari, E.; Kucukboyaci, N.; Toker, G. Neurobiological Evaluation of Thirty-One Medicinal Plant Extracts Using Microtiter Enzyme Assays. *Clin. Phytoscience* **2017**, 2(1), 1–12. DOI: 10.1186/s40816-016-0023-6.

[15] Heglmeier, A.; Zidorn, C. Secondary Metabolites of Posidonia Oceanica (Posidoniaceae). *Biochem. Syst. Ecol*. **2010**, 38(5), 964–970. DOI: 10.1016/j.bse.2010.07.001.

[16] Cuny, P.; Serve, L.; Jupin, H.; Boudouresque, C. F. Water Soluble Phenolic Compounds of the Marine Phanerogam Posidonia Oceanica in a Mediterranean Area

Colonised by the Introduced Chlorophyte Caulerpa Taxifolia. Aqua*t. Bot.* **1995**, 52(3), 237–242. DOI: 10.1016/0304-3770(95)00504-8.

[17] Kittaka, K. Relationship between Wind Direction and Air Temperature in the Osaka Center City Determined Using Fixed Point Observation. *Technol. Reports Kansai Univ.* **2014**, 9, 1–5

[18] Bitam, F.; Ciavatta, M. L.; Manzo, E.; Villani, G.; Gavagnin, M. The First Record of Neolignans from the Marine Phanerogam Posidonia Oceanica. *Phytochem. Lett.* **2012**, 5(4), 696–699. DOI: 10.1016/j.phytol.2012.06.016.

[19] Sica, D.; Piccialli, V.; Masullo, A. Configuration at C-24 of Sterols from the Marine Phanerogames Posidonia Oceanica and Cymodocea Nodosa. *Phytochemistry* **1984**, 23(11), 2609–2611. DOI: 10.1016/S0031-9422(00)84109-6.

[20] Hammami, S.; Salem, A. Ben; Ashour, M. L.; Cheriaa, J.; Graziano, G.; Mighri, Z. A Novel Methylated Sesquiterpene from Seagrass Posidonia Oceanica (L.) Delile. *Nat. Prod. Res.* **2013**, 27(14), 1265–1270. DOI: 10.1080/14786419.2012.725401.

[21] Jerković, I.; Marijanović, Z.; Roje, M.; Kus, P. M.; Jokić, S.; Čož-Rakovac, R. Phytochemical Study of the Headspace Volatile Organic Compounds of Fresh Algae and Seagrass from the Adriatic Sea (Single Point Collection). *PLoS One* **2018**, 13(5), 1–13. DOI: 10.1371/journal.pone.0196462.

[22] Elabbar, F.; Alasply, A. GC-MS Analysis of n-Hexane Extracts of Marine Seagrass Posidonia Oceanica Leaves , Rhizomes and Roots Collected from Benghazi Beach Libya. *Scientiae Radices* **2024**, 3(1), 1–8 DOI: 10.58332/scirad2024v3i1a01.

[23] Cheng, K.; Wang, X.; Zhang, S.; Yin, H. Discovery of Small-Molecule Inhibitors of the TLR1/TLR2 Complex. *Angew. Chemie - Int. Ed.* **2012**, 51(49), 12246–12249. DOI: 10.1002/anie.201204910.

[24] Sinapic acid - Optional[13C NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/LHD9O4J5Zyl (accessed 2024-06-25).

[25] Sinapic acid - Optional[1H NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/DDyS82fcp73 (accessed 2024-06-25).

[26] α-Methylcinnamic acid - Optional[1H NMR] - Chemical Shifts - SpectraBase. https://spectrabase.com/spectrum/3isGDRKLyYh (accessed 2024-06-25).

[27] α-Methylcinnamic acid - Optional[13C NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/Cth7ZobvqJ5 (accessed 2024-06-25).

[28] Acetosyringone - Optional[1H NMR] - Chemical Shifts - SpectraBase. https://spectrabase.com/spectrum/3c4P8WSZxZ2 (accessed 2024-06-25).

[29] Acetosyringone - Optional[13C NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/GCmKnuqPGun (accessed 2024-06-25).

[30] Chlorogenic acid - Optional[1H NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/AvuCyU3ZsMr (accessed 2024-06-25).

[31] Chlorogenic acid - Optional[13C NMR] - Spectrum - SpectraBase. https://spectrabase.com/spectrum/94typQ1fAr (accessed 2024-06-25).

[32] 4-Ethoxycoumarin - Optional[1H NMR] - Chemical Shifts - SpectraBase. https://spectrabase.com/spectrum/IguxabvKoeq (accessed 2024-06-25).

[33] 4-Ethoxycoumarin (35817-27-7) 13C NMR spectrum. https://www.chemicalbook.com/SpectrumEN_35817-27-7_13CNMR.htm (accessed 2024-06-25).

[34] bmse000607 Syringic Acid at BMRB. https://bmrb.io/metabolomics/mol_summary/show_data.php?id=bmse000607&whichTab=1 (accessed 2024-06-25).

[35] Hasdiana, U. Extraction_technologies_for_medicinal_and_aromatic_plants. *Anal. Biochem.* **2018**, 11(1), 1–5.

[36] Sheel, D. R.; Nisha, K. Qualitative Phytochemical Analysis for Isolation of Terpens from Clerodendron Infortunatum Leaves. *IOSR J. Appl. Chem.* **2014**, 7(7), 14–18. DOI: 10.9790/5736-07721418.

[37] Van Dijken, J. W. V.; Ruyter, I. E. Surface Characteristics of Posterior Composites after Polishing and Toothbrushing. *Acta Odontol. Scand.* **1987**, 45(5), 337–346. DOI: 10.3109/00016358709096356.

[38] Mebrat, Y. A Laboratory Manual for the Preparation of Chemical Reagents, Solutions and Special Indicators. Cogniz. *J. Multidiscip. Stud.* **2021**, 1(12), 1–62. DOI: 10.47760/cognizance.2021.v01i12.001.

[39] Oshadie, G.; Silva, D.; Abeysundara, A. T.; Minoli, M.; Aponso, W. Extraction Methods, Qualitative and Quantitative Techniques for Screening of Phytochemicals from Plants. Am. J. Essent. *Oils Nat. Prod*. **2017**, 5(2), 29–32.

[40] R, R. M. Preliminary Phytochemical Screening and Antioxidant Activity of an Ayurvedic Formulation: Balarishtam. *Int. J. Res. Ayurveda Pharm*. **2011**, 2(6), 1645–1647. DOI: 10.1111/j.1753-4887.1998.tb01670.13.

[41] Raal, A.; Meos, A.; Hinrikus, T.; Heinämäki, J.; Romāne, E.; Gudienė, V.; Jakštas, V.; Koshovyi, O.; Kovaleva, A.; Fursenco, C.; Chiru, T.; Nguyen, H. T. Dragendorff's Reagent: Historical Perspectives and Current Status of a Versatile Reagent Introduced

over 150 Years Ago at the University of Dorpat, Tartu, Estonia. *Pharmazie* **2020**, 75(7), 299–306. DOI: 10.1691/ph.2020.0438.

[42] Dubale, S.; Kebebe, D.; Zeynudin, A.; Abdissa, N.; Suleman, S. Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia*. J. Exp. Pharmacol.* **2023**, 15, 51–62. DOI: 10.2147/JEP.S379805.

[43] Lee, H.; Oh, C.; Kim, S.; Dey, D. K.; Kim, H. K.; Bajpai, V. K.; Han, Y. K.; Huh, Y. S. Metasequoia Glyptostroboides Potentiates Anticancer Effect against Cervical Cancer via Intrinsic Apoptosis Pathway. *Sci. Rep.* **2021**, 11(1), 1–9. DOI: 10.1038/s41598-020-79573-8.

[44] Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* 1**978**, 43(14), 2923–2925. DOI: 10.1021/jo00408a041.

[45] Vogt, T. Phenylpropanoid Biosynthesis. *Mol. Plant* **2010**, 3(1), 2–20. DOI: 10.1093/mp/ssp106.

[46] De Paula E Silva, A. C. A.; De Oliveira, H. C.; Scorzoni, L.; Marcos, C. M.; Dos Santos, C. T.; Fusco-Almeida, A. M.; Guerta Salina, A. C.; Medeiros, A. I.; Almeida, F.; Li, S. C.; Boone, C.; Mendes-Giannini, M. J. S. Decyl Gallate as a Possible Inhibitor of N-Glycosylation Process in Paracoccidioides Lutzii. *Antimicrob. Agents Chemother.* **2019**, 63(11). DOI: 10.1128/AAC.01909-18.

[47] Tine, Y.; Renucci, F.; Costa, J.; Wélé, A.; Paolini, J. A Method for LC-MS/MS Profiling of Coumarins in Zanthoxylum Zanthoxyloides (Lam.) B. Zepernich and Timler Extracts and Essential Oils. *Molecules* **2017**, 22(1) 174. DOI: 10.3390/molecules22010174.

[48] Murakami, A.; Gao, G.; Kim, O. K.; Omura, M.; Yano, M.; Ito, C.; Furukawa, H.; Jiwajinda, S.; Koshimizu, K.; Ohigashi, H. Identification of Coumarins from the Fruit of Citrus Hystrix DC as Inhibitors of Nitric Oxide Generation in Mouse Macrophage Raw 264.7 Cells. *J. Agric. Food Chem.* **1999**, 47(1), 333–339. DOI: 10.1021/jf980523e.

[49] De Paula E Silva, A. C. A.; Costa-Orlandi, C. B.; Gullo, F. P.; Sangalli-Leite, F.; De Oliveira, H. C.; Silva, J. D. F. Da; Scorzoni, L.; Pitangui, N. D. S.; Rossi, S. A.; Benaducci, T.; Wolf, V. G.; Regasini, L. O.; Petrônio, M. S.; Silva, D. H. S.; Bolzani, V. S.; Fusco-Almeida, A. M.; Mendes-Giannini, M. J. S. Antifungal Activity of Decyl Gallate against Several Species of Pathogenic Fungi. *Evidence-based Complement. Altern. Med.* **2014**, 506273. DOI: 10.1155/2014/506273.

[50] De Paula E Silva, A. C. A.; De Oliveira, H. C.; Scorzoni, L.; Marcos, C. M.; Dos Santos, C. T.; Fusco-Almeida, A. M.; Guerta Salina, A. C.; Medeiros, A. I.; Almeida, F.; Li, S.

C.; Boone, C.; Mendes-Giannini, M. J. S. Decyl Gallate as a Possible Inhibitor of N-Glycosylation Process in Paracoccidioides Lutzii. Antimicrob. *Agents Chemother*. **2019**, 63 (11). DOI: 10.1128/AAC.01909-18.

[51] Ampath, N. S.; Ravindhan, S. A.; Onnuswamy, M. N. P.; Aisankar, P. J.; Rinivasan, P. C. S. X-Ray Structure Analysis Online Crystal Structure of 1-Phenylsulfonyl-2-Methyl-3-Cyanoindole. *Anal. Chem.* **2005**, 21, 69–70

[52] Nićiforović, N.; Abramovič, H. Sinapic Acid and Its Derivatives: Natural Sources and Bioactivity. *Compr. Rev. Food Sci. Food Saf.* **2014**, 13(1), 34–51. DOI: 10.1111/1541-4337.12041.

[53] Gil, M.; Wianowska, D. Chlorogenic Acids – Their Properties, Occurrence and Analysis. *Ann. Univ. Mariae Curie-Sklodowska, Sect. AA – Chem*. **2017**, 72(1), 61. DOI: 10.17951/aa.2017.72.1.61.

[54] Srinivasulu, C.; Ramgopal, M.; Ramanjaneyulu, G.; Anuradha, C. M.; Suresh Kumar, C. Syringic Acid (SA) – A Review of Its Occurrence, Biosynthesis, Pharmacological and Industrial Importance. *Biomed. Pharmacother.* **2018**, 108, 547–557. DOI: 10.1016/j.biopha.2018.09.069.