EUROPEAN JOURNAL OF BIOMEDICAL
AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 12
Year: 2024

BIOACTIVE COUMARIN FROM METHANOL EXTRACT OF SEED TRACHYSPERMUM STICTOCURPUM LINN (AAJMODA SEED)

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Article Received on 16/10/2024 **Article Revised on 06/11/2024** **Article Accepted on 26/11/2024**

ABSTRACT

Trachyspermum species are herbaceous plants belongs to *Apiceae* family. Their seeds are used as folklore medicine from very antiquity to cure different types of diseases reported in Indian traditional system of medicine Ayurveda. The seeds also used as active ingredient for preparation exotic spices. The phytochemical investigation of methanol extract of the seeds of *Trachyspermum stictocurpum* revealed the presence of lactone class of compounds known as pyrano-coumarin and furanocoumarin as major chemical ingredients. In literature, these class of compounds are mainly used as skin infections such as psoriasis, leukoderma etc. with the help of photo dynamic therapy (PDT).

KEYWORDS: Coumarins; seeds; T. stictocarpum; isolation and structural characterization; XRD study.

INTRODUCTION

Trachyspermum species are annual or biennial herbaceous plant belong to *Apicaece* family. Amongst them, *Trachyspermum ammi* (aajwan), *Trachyspermum stictocarpum* (aajmoda), *Trachyspermum roxburghanium* (randhuni) are widely cultivated in India due to their medicinal properties and used as spices in different Indian cuisine.[1-3] The *Trachysperemum stictocurpum* also known as *Apium graveollens* Linn in another botanical name previously. The genus *Trachyspermum* are not only valued for their traditional medicinal properties, but also extensively use as spices in domestic cooking and also used as folklore remedial agent to cure different kind of diseases, as an active ingredient for preparation of exotic spices in various Indian cuisines, health care, health promoting purposes etc.[3-5] Amongst the different *Trachyspermum* species most of the scientific investigations have been carried out with *T. ammi* (aajwan). The seed extract of *T. ammi* exhibits anti-spasmodic,[5] chemo-preventive,[6] anti-inflammatory,[7-10] anti-microbial,[11,12] anti-ulcer,[13] anti-bacterial[13] anti-fungal,[14, 15] stimulant activities,[14] anti-oxidant,[16-21] cardiovascular activity,[22] anti-cancer activity,[23-33] neuroprotector,[34] molluscidal activity,[35-39] leukoderma,[40-41] anti-leprosy[42] and is prescribed as a household remedy for indigestion due to over eating. Besides, the seed extract is reported to possess a lot credential pharmacological activities such as anti-bacterial,[13] anti-fungal,[14,15] anti-oxidants,[16-21] anti-cancer,[23-33] natural antacid,[13] and molluscidal activities.[35-39] The seed extract is also used in pickle, bakery & confectionary and pan mixtures due to its pleasant aroma.[1-5] The steam volatile of *T. ammi* seeds have been investigated extensively and variations in the chemical constituents are reported in different varieties. (ref). The major constituent of the steam volatile of *Trachyspermum ammi* is a phenol known as thymol.[43,44]

The seeds of *Trachyspermum stictocurpum* called as Ajmod in local dialect Hindi and in celery seed in English. Their seeds are morphologically very similar to Trchyspermum ammi (Ajwan) and Trachyspermum roxburghanium (Randhuni). Chemical profiling of methanol extract *T. stictocurpum* revealed the major chemical constituents are coumarins. This class of secondary metabolites are very useful for treatment psoriasis/leukoderma using photo dynamic therapy (PDT). A series of coumarin and their glycosides have been isolated from methanol extract of the seed of *T. stictocarpum* by column chromatography over silica gel with gradient solvent elution by using a binary mixture of ethyl acetate in hexane followed by a mixture of methanol in chloroform. The structures of the compounds have been established in combination with ^{1}H NMR, ^{13}C NMR, EIMS and in some cases confirmed by X-ray diffraction. On acid hydrolysis of glycoside, the sugar moiety has been identified as β-pyran glucosyl by circular paper chromatography and GCMS analysis of its acetate derivative on comparison with respect to acetate of authentic sugar sample.

RESULTS AND DISCUSSION

The plant *T. stictocarpum* is well known herbal spice plant widely distributed all over India. It has recognized for its folklore botanical medicinal uses due to its high pharmacological potentials against various kind of ailments and as an active ingredient for preparation of exotic spices in Indian cuisine.[1-5] In local dialect, the seed of *Trachyspermum stictocarpum* known as ajmod/ajmoda. This plant has been cultivated for seeds that are commonly used as a spice, condiment because of its flavor and fragrance. In order to investigate the bioactive constituents, present in the seed of *Trachyspermum stictocurpum*, the best quality of seeds has been purchased from local herbal market Pydhuni in Mumbai in May 2019. The seeds were powdered finely and extracted with methanol at room temperature. The extract was evaporated using rota-vapor at reduced pressure maintaining temperature of water bath at 40 ^{0}C to obtain a brown sticky mass. This crude extract was fractioned by column chromatography over silica gel with gradient solvent elution by using a binary mixture of n-hexane ethyl acetate followed by methanol-chloroform to yield several fractions. Each fraction was monitored by TLC. The fractions with similar TLC profiles were combined, and in some cases, further purified by open column chromatography, gel permeation chromatography (GPC) over sephadexLH20, high performance (HPLC) and preparative TLC followed by crystallization to afford respective products. The color teste, spray reagent teste, UV absorption spectrum of these compound are very closely resembled with absorption in the range of 243-334 nm indicating presence of similar type of chromophore. Spraying with 10% aqueous H_2SO_4 on TLC plate followed by heating at 120^{0}C for 5 min showed a yellow spot which is indication of coumarin class of compound.[46-49] This has led to the new finding of the presence of medicinally important coumarins like seselin, dihydroseselin, coumarin, psoralene, pimpinellin, isopimpinellin and bergapten that are known to possess impressive anti-tumor,[27-28] anti-cancer,[23-33] leukoderma,[40-41] anti-leprosy activities.[42] The structures of the compounds have been determined in combination with ^{1}H NMR, ^{13}C NMR, EIMS and in some cases confirmed by X-ray diffraction study. On acid hydrolysis of glycoside obtained aglycone and sugar. The sugar moiety has been identified as β-pyran glucosyl by NMR study, circular paper chromatography and GCMS analysis of its acetate derivative on comparison with respect to authentic sugar sample. The present paper deals with the isolation and structure elucidation of prenylated glycol-coumarin along with other known coumarins. This seed may be used as abundant natural resources of this class of compounds. The chemical structure of major coumarins isolated from methanol extract of seed of *Trachyspermum stictocarpum* are depicted below.

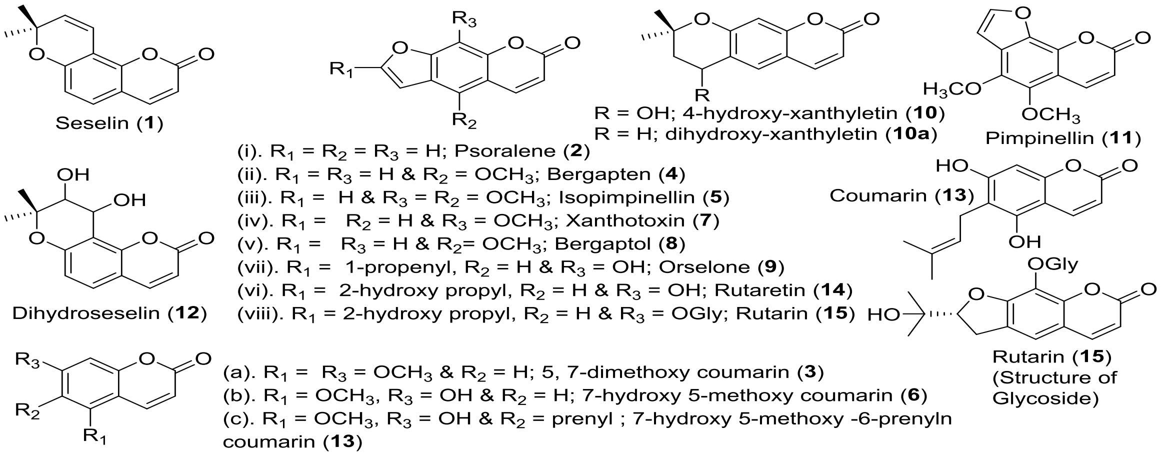

Figure 1: Chemical structure of coumarins isolated from *trachyspermum stictocarpum*.

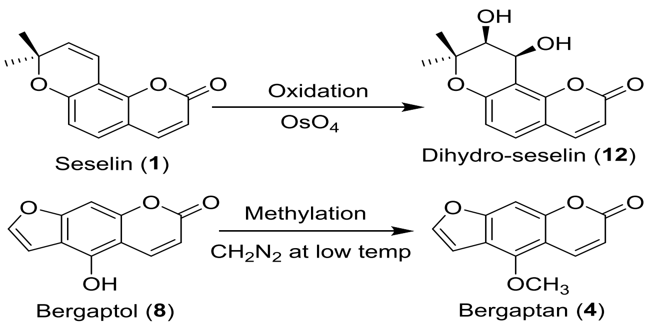

Figure 2: Chemical transformation of bergaptol to bergapten using diazomethane.

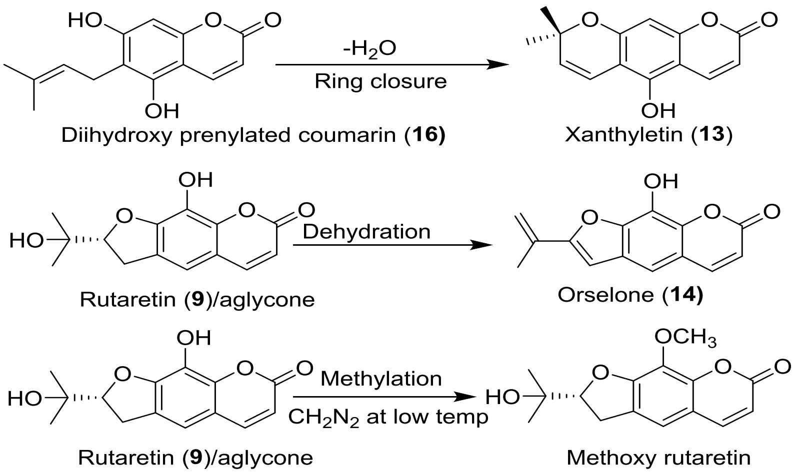

Figure 3: Chemical transformation of bergaptol to bergapten using diazomethane.

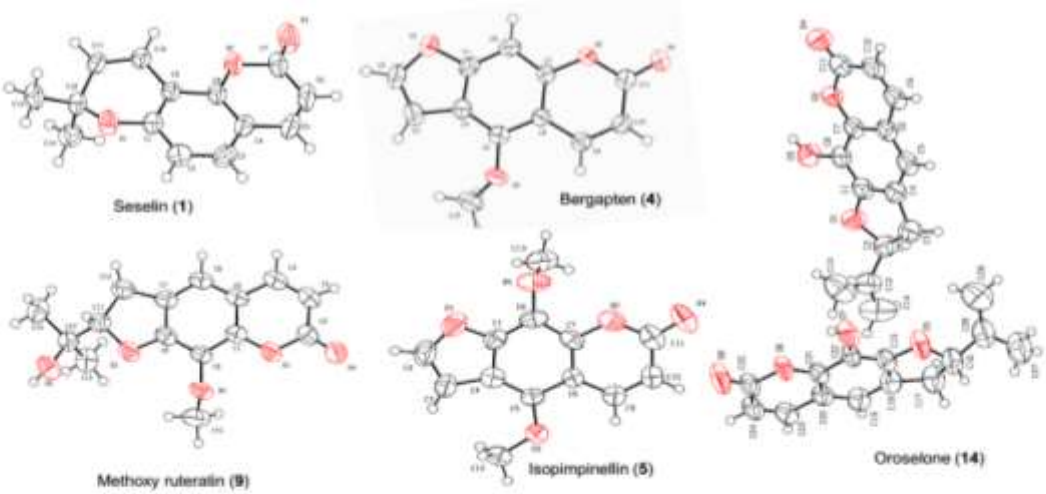

Figure 4: Crystal structure of few coumarins isolated from *Trachyspermum stictocapum*.

Expermental section

General information

Melting points were determined using a Buchi M560 point apparatus. Specific rotations were obtained using a JASCO DIP 1000 digital polarimeter. UV spectra were measured on a Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker Avance 200, Varian 500 MHz, Varian 600 MHz, Varian 800 MHz spectrometers using residual $CHCl_3$/HDO as an internal standard. Chemical shifts are given in ppm (δ_C & δ_H), relative to residue $CHCl_3$/HDO (7.25 & 77.00/4.78 or 3.30 & 49.00 ppm). Mass spectra were recorded using Fission 8000 (8000 series, UK) and Shimadzu QP5050A mass spectrometer, Japan. UHPLC/MS analysis of the samples was carried out using a Vanquish UHPLC system coupled to a Q-Exactive quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific, USA) at the Ohio State University, College of Pharmacy Instrumentation Facility. Silica gel 60 (Merck) was used for analytical TLC. Silica gel (230-450 mesh, Aldrich, USA) was used for column chromatography. All compounds were visualized in TLC under exposure of both long range as well as short range wavelength, using vanillin-perchloric acid-EtOH followed by heating at 110 °C for 5 min and neutral $FeCl_3$ in MeOH.

Plant material

The seed of *Trachyspermum stictocarpum* was purchased from local herbal and spice market Pydhuni, Mumbai in May, 2019. The material was authenticated by Dr. Hussian Barbhuiya, Landscape and Cosmetic Section, A & SED Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085.

Extraction and Isolation

Freshly dried seeds (100 gram) of *Trachyspermum stictocarpum* were powdered using mechanical grinder and extracted with analytical grade methanol (3x250 mL) at room temperature to obtain methanol extract. Solvent was removed from methanol extract using rota-vapor at reduced pressure maintaining water bath temperature at 40 °C to afford a brown viscous residue (~1.5 g). For preliminary investigation to check chemical composition of methanol extract, trace amount of residue was dissolved in methanol and monitored by micro TLC plate. This microplate was developed using different combinations of solvent systems, sprayed with different reagents, on exposure of UV radiation, several spots were visualized. Based on this idea of solvent system used on microplate, the solvent system chosen for column chromatography was set up accordingly.

This extract was fractionated by column chromatography over silica gel (500-gram, 230-400 mesh, Aldrich, USA) and eluted with a step gradient of hexane, ethyl acetate in hexane, then chloroform and mixtures of methanol in chloroform to obtain thirty fractions, with the volume of each aliquot being 20-50 mL. In some cases, the volume of the aliquot collected was more than 50 mL. Fractions were monitored by TLC to examine the chemical compositions of the respective fractions. The fractions with similar chemical profiles were combined and, in some cases, further purified by using repetitive column chromatography on an open column chromatography over silica gel, and gel permeation chromatography (GPC) using sephadexLH20 with gradual solvent elution followed by preparative thin layer chromatography (PTLC).

Fraction 1 and 2 were eluted with 5-10% ethyl acetate in petroleum ether. Both the fractions were monitored by TLC. TLC profiles indicated that they were similar in composition and combined. A residue (~ 98.7 mg) has been obtained to removing the solvent from combined fractions. Preliminary investigations revealed that it was a mixture of few terpenoids. Major constituent of it was β salinene (50%) contaminated with small amount of globulol (12%) dihydrocarveol acetate (14.8%), ledenoxides (15%), dihydrocarveol (10.9%), caryophylene oxide (12.4%) analyzed by GCMS.

Fraction 3 was eluted with 15% ethyl acetate in petroleum ether. The volume of collected aliquot was approximately 150 ml. On removing solvent, a pale-yellow sticky mass was obtained. This sticky mass was analyzed by GLC and GCMS analysis. It revealed that it was mixture of methyl ester stearic (25%), palmitic (25%), oleic (30%), linoleic (10%) and a trace amount linolenic acid (5%) on compare data with respect to authentic samples.

Fraction 4 was eluted with 15-20% ethyl acetate in petroleum ether. Total volume of each collected aliquot was approximately 150 ml. It was subjected to concentrate in a small volume for crystallization. A pale-yellow color crystal separated to filter through a micro filter system and washed thoroughly using cold 10% ethyl acetate in petroleum ether for three times to obtain crystals (2 g, abundance 2% dry seed weight), mp 120 $^{\circ}$C (lit.119-120 $^{\circ}$C).[22-24] In ^{1}H NMR spectrum, three pair of doublets appeared at 7.57, 6.21 with coupling constant value J equal to 9.4 Hz corresponds H-4 and H-3, 7.19, 6.70 with coupling constant 8.6 Hz corresponds H-6 and H-8 and 6.87, 5.71 having coupling constant 10.2 Hz corresponds H-9, H-10 respectively. It showed UV absorption 242, 253, 327 nm. This compound was identified as seselin (**1**) in in compare with spectral data available in literature.[50-55] Finally, structure of the compound has been confirmed by single crystal X-ray diffraction study.[56] The major constituents of the mother liquor were an aliphatic compound oleyl ester of oleic acid a pale-yellow sticky mass contaminated with very trace quantity psoralene (**2**),[57-60] and characterized in compare with the authentic sample. The other coumarin separated by preparative TLC was shown a pale green coloration with ferric chloride and a (+)-ve indo-phenol reaction indicating free para position to the phenolic hydroxy group.[48-49] It methylated with diazomethane gives crystallized product (10% ethyl acetate in petroleum ether) possesses mp 145-146^{o}C and found to be identical with 5, 7-dimethoxy coumarin (**3**) (lit.146-147°C) with compare the spectral data available in literature.[57-60] Based on the above evidences the parent compound was identified as 7-methoxy-5-hydroxy coumarin (abundance ~ 0.011 mg/g) possesses mp 237-238°C.[61-63] Fraction 5 was eluted with 20% ethyl acetate in petroleum ether and volume of the collected aliquot was approximately 150 ml. A colorless solid (~ 81.0 mg) was obtained on while concentrating in small volume. It was re-crystallized (15% ethyl acetate in petroleum ether) to give needle shaped crystals possesses mp 187-191°C. It showed UV absorption 221, 247, 266 and 306 nm. In ^{1}H NMR spectrum, it gave a pair doublet appeared at 6.25 and 8.14 (H-4) with coupling constant 10 Hz indicating H-3 and H-4 protons; furan ring doublets at 7.59 and 7.02 with coupling constant 2.3 Hz presenting to H-2′ and a singlet at 7.12 (H-8) respectively. A sharp singlet integrated 3H for aromatic methoxy group located at 4.25 ppm. The spectral data the isolated compound (4) was identified as bergapten in compare with published spectral values reported in literature.[61-63] Therefore, isolated product was bergapten. Mother liquor was consisting few constituents which purified by preparative TLC. The analysis is incomplete on account of low yield of the isolated products.

Fraction 6 was eluted with 20-22% ethyl acetate in petroleum ether and total volume of collected aliquot was approximately 200 ml. It was allowed to slow evaporate at room temperature for crystallization. A white crystalline substance was appeared and separated by filtration. The crystals were washed with cold 10% ethyl acetate in petroleum ether for thrice. TLC analysis in a micro TLC plate revealed that it was a mixture of two compounds (1:1) with very close R_f values. These were separated by preparative TLC with multiple developments and eluted with chloroform. Individual residues were obtained on removable of solvents and crystallized from 10% ethyl acetate in petroleum to afford a needle shaped crystal for both the compounds. The first compound having comparatively slightly higher R_f value and identified as bergapten (**4**).[57-65]

The second compound possessed mp 143 ^{o}C, possessed UV absorption 221, 240, 266-272 and 311 nm. In the ^{1}H NMR, the signals appeared two doublets at 6.25 and 8.08

with coupling constant 10 Hz each integrating one proton corresponds to H-3 and H-4; another pair of doublets appeared at 7.59 (H-2′) & 6.96 (H-3′) with coupling constant 2.3 Hz showed the presence of un-substituted furan rings and two sharp singlets due to aromatic methoxy groups integrating three protons each exhibited signals at 4.04 and 4.15 ppm. The physical, chemical and spectral data of the isolated compound (**5**) (abundance ~ 0.013 mg/g) was identical in all respect with authentic pimpinellin (**5**) in comparison with spectral data available in literature and confirmed by single crystal XRD study.[65] Major constituents of mother liquor of this fraction were separated by preparative TLC identified as methyl ester of oleic acid, bergapten (**4**), 7-hydroxy-5-methoxy-coumarin (**6**), mp 278-280 °C as explained previously.[57]

Fraction 7 was eluted with chloroform and the volume of each aliquot was approximately 150 ml. A mixture of an amorphous solid was obtained to remove the solvents and TLC analysis in micro plate revealed that it was a mixture of three components, separated by preparative TLC. These compounds were identified bergapten (**4**), pimpinellin (**5**) and 7-hydroxy-5-methoxy coumarin (**6**) by comparing both physical and spectral data as discussed above.[57-65]

Fraction 8 was eluted with 5% MeOH in $CHCl_3$ and volume of collected aliquot was approximately 150 ml. A sticky mass (~20 mg) was obtained to removing the solvents and its analysis was incomplete.

Fraction 9 was eluted with 10% MeOH in chloroform and collected volume of aliquot was 150 ml. It was allowed to crystallize to slow evaporation for two days in fume-hood. It was filtered using micro filter and re-crystallized using 10% ethyl acetate in petroleum gave crystals of xanthotoxin (**7**) (~ 88 mg, abundance ~ 0.44 mg/g), mp 145-146 °C (lit. 145-146 °C).[57-65] It exhibited UV absorption at 221, 242, 247, 260 and 297 nm. In ^1H NMR spectrum, the signals were appeared as a pair of doublets at 6.32 and 7.72 integrated one proton each indicating H-3 and H-4; a singlet at 7.31 due to H-5; a pair doublet appeared at 7.65, 6.78 integrating one proton with coupling constant, 2.3 Hz presenting H-2′ and H-3′. A sharp singlet at 3.25 integrating 3H for aromatic-OCH_3 group. The structural determination of compound (**7**) has been conducted by spectroscopic methods in comparison with published spectral data available in literature and identified as xanthotoxin (**7**).[57-65] The mother liquor obtained on crystallization was combined to next fraction as they are similar in composition to monitoring by TLC.

Fraction 10 was eluted with 15-20% MeOH in chloroform. The solvent was removed of these combined fractions to afford a residue, a mixture of two constituents to monitoring by TLC and it purified by preparative TLC (7% EtOAc in petroleum ether) and crystallized from 10% ethyl acetate in petroleum ether and 5% MeOH in chloroform respectively both afforded needle shape crystals The first compound was readily soluble in dilute alkalis, shown pale green color on spray with ferric chloride solution, mp 277-279 °C (lit. 275-278 °C). On methylation with diazomethane gave an ether possesses mp 187-189 °C and showed UV absorption 243, 250, 263, 269 and 313 nm and identified as bergapten (**7**). Therefore, parent phenolic compound was bergaptol (**8**).[57-65] Second compound was yellowish white crystalline solid possesses mp 187-188 °C.[57] Molecular formula of the compound has determined as $C_{14}H_{14}O_4$ based on EIMS analysis. It showed dark coloration on sprayed with neutral ferric chloride on TLC plate, characteristic UV absorption and treatment with alcoholic KOH gave positive test indicated that it was a coumarin containing phenolic hydroxyl group.[46-49] It afforded a solid of methyl ether of it on treating with diazomethane in ether which re-crystallized from 10% ethyl acetate to get light yellow prism shaped crystals, mp 146-147 °C. A sharp singlet appeared in ^1H NMR at 3.92 ppm integrated 6H due to two aromatic methoxyl groups indicated the attachment of it at C-5 and C-7 of the coumarin moiety.[46-49] It showed a pair of doublets at 6.10 and 7.98 ppm contents one proton each with coupling constant, *J* equal to 9.5 Hz indicating protons located at position C-3 and C-4 of the coumarin. A sharp singlet at 3.92 contents 6H corresponding two methoxyl groups, a double doublet at 2.90 ppm integrated two protons was assigned to the benzylic methylene and a quartet at 3.58 ppm was due to methine proton. The deuterium exchangeable signal at 2.3 ppm belongs to the hydroxyl proton. The singlet at 1.3 ppm (6H, $2xCH_3$) corresponds to the gem-methyl of the prenylated moiety. The shapes, positions and protons content described as above was pin point the prenylated side chain attached at either at the position C-8 or at C-6 undergo ring closure giving rise furanocoumarin as being prenyl chain ring closure occurring in many natural products.[48-49] Negative Gibbs test, chemical shift value of the particular proton in ^1H NMR spectrum indicated that the attachment of the prenyl chain ring closure with C7-oxygen atom of the coumarin form fuano moiety was at C-6 not at C-8.[46-49] Based on the above spectral evidences, the structure of the compound assigned as a furanocoumarin named by ruteratin (**9**). Finally, the structural confirmation of furanocoumarin had been carried out by single crystal X-ray study (transformed as methyl derivative).[65]

Fraction 11 was eluted with solvent system 20-25% MeOH in chloroform. The volume of aliquot was 200 mL. On removal of the solvents, a dark brown sticky mass was obtained. It was highly hygroscopic in nature. It was purified by means of gel permeation chromatography over sephadexLH20 eluting with a binary mixture of solvent, 10% methanol in chloroform. Six fractions were collected consisting volume of each aliquot approximately 50 ml. The first fraction contained polymeric materials as major constituent was discarded and residues obtained from other fractions were negligible except second fraction. Analysis of these residues obtained from other fractions was incomplete on account of low yield. The constituents eluted in second fraction were a mixture of few components and purified by preparative TLC (15% MeOH in chloroform) to separate six bands. Out of six bands first and second bands were in major amount, others were present in minor quantities. The residue obtained from first band was purified on repeated preparative TLC on multiple solvent developments (25% ethyl acetate in petroleum ether) afforded four pure compounds coded as R-1, R-2, R-3 and R-4 and their identification was done as follows.

***Analysis of R-1*:** This amount of the isolated phenol (~5 mg) was in a very less, mp 274-276 °C (lit. 276-278 °C) and formed a methyl ether with diazomethane in ethereal solution possesses mp185-187 C (lit. 187 °C) and found to be identical with bergapten. Therefore, isolated parent compound was identified as bergaptol.[8]

***Analysis of R-2*:** An amorphous powder isolated as minor product, possesses mp 187 °C. In ^{1}H NMR spectrum, a pair of doublets appeared at δ 7.61 and 6.85 ppm with coupling constant 9.5 Hz integrating one proton each belongs to coumarin moiety. A singlet at 6.85 integrating one proton due to aromatic proton, a double doublet at 5.36 with *J* values 8.8 & 8.7 Hz integrated one proton, two D_2O exchangeable protons at 5.12 and 4.96 due both alcoholic and phenolic-OH groups, a pair doublet appeared at 3.35 and 3.13 as double doublet integrating one proton each with coupling constant 8.7 and 16.4 Hz probably due to H_a-3′ & H_b-3′, a pair of singlet appeared at 1.78 and 1.70 integrating 3H each due gem methyl of chromene moiety. Based on the above evidences, the compound was characterized as 4′ hydroxy xanthyletin.[10]

***Analysis of R-3*:** A pale yellow needle shaped crystal possesses mp 147-148 °C (lit. 147-146 °C); It exhibited UV absorptions at 221, 240, 247, 268, 311 nm. In ^{1}H NMR spectrum, the signals appeared at 6.25, 8.10 ppm as doublets with coupling constant 10 Hz integrating one proton each indicating H-3 and H-4. Another couple of doublets appeared at 7.59, 6.98 ppm with coupling constant 2.2 Hz representing H-2′, H-3′ of un-substituted furan ring. A sharp singlet appeared at 4.18 ppm integrating 6H due to two aromatic methoxyl groups. Since spectral data of isolated product was in accord with authentic pimpinellin in all respects, therefore R-3 was identified as pimpinellin.[11] The spectrum is much similar to compound[5] which is linear isomer of compound.[11]

***Analysis of R-4*:** It was identified as xanthotoxin[7] by comparing data with authentic samples as discussed above.

The second band seem to be a mixture of at least three components, showed very close R_f indicated on multiple development of preparative TLC plate using 20% ethyl acetate in petroleum ether to separate three bands coded as S-1, S-2 and S-3 respectively and they were identified as follows-

(i) *Analysis of S-1*

Colorless crystalline substance having mp 186-188 °C (~15 mg, abundance 0.075 mg/g).[66-67] Molecular formula of the compound has determined as $C_{14}H_{14}O_8$ by HRMS. It did not show coloration on sprayed with neutral alcoholic ferric chloride on TLC plate whereas shown purple/yellow/royal blue color on spray with anisaldehyde/10% aqueous H_2SO_4/ vanillin alcoholic solution followed strong heating at 120 °C for 5 min.[46] UV absorption spectra of the compound close resembled to the spectra of coumarin. Total three pairs doublet and a pair singlet appeared in ^{1}H NMR spectra described as an AB quartet centered at 6.17 and 7.64 ppm contents one proton each with coupling constant 9.4 Hz due to chromene moiety, a pair of ortho doublet at 7.3 and 6.77 ppm with coupling constant 8.6 Hz due to aromatic protons, a pair doublet comparatively located at upfield at 4.9 and 3.83 ppm contents one proton with coupling constant 8.4 Hz indicated protons attached with carbon bearing oxygen atoms probably these were *cis* glycol system. Two singlets appeared at 1.5 and 1.3 ppm integrating 3H each due to gem di-methyl of the chromene moiety. Based on the above spectral evidences, the structure of the compound was assigned as dihydroxy seselin.[12]

(ii) *Analysis of S-2*

Molecular formula of the compound has determined as $C_{14}H_{14}O_4$ based on EIMS, possesses mp 148 °C It showed dark coloration on spray with neutral ferric chloride on TLC plate, characteristic UV absorption and treatment with alcoholic KOH indicated that it was a coumarin containing phenolic hydroxyl group.[46-49] It methylated with diazomethane in ether to afford methyl ether which shown a sharp singlet appeared at 3.92 ppm integrated 6H in ^{1}H NMR indicated that it was dimethoxy derivative attached at C-5 and C-7 of coumarin moiety.[50-55] It shown a pair of doublets at 6.10 and 7.98 ppm contents one proton each with coupling constant 9.5 Hz characteristics of protons at position C-3 and C-4 of coumarin, a triplet at 2.90 ppm (2H) assigned due to the benzylic methylene and a quartet at 3.58 ppm was due to methine proton. The deuterium exchangeable signal at 2.3 ppm belongs to the two hydroxyl protons. The singlet at 1.3 ppm (6H, 2x-CH_3) corresponds to the gem-methyl. The shapes, positions and proton content described as above was pin point the side chain attached at either at the position C-8 or at C-6 as being prenyl chain occurring in many natural products.[46-47] Negative Gibbs test & chemical shift value indicated that the attachment of the prenyl chain was at C-8 not at C-6. Based on the above evidences the structure of the compound was assigned as 5, 7-dihydroxy 6-prenyl coumarin.[13]

(iii) Analysis of S-3[14]

It showed a pair of doublets at 6.10 and 7.98 ppm contents one proton each with coupling constant 9.5 Hz indicating protons position at C-3 and C-4 of coumarin. A sharp singlet at 6.85 ppm integrated one aromatic proton, a double doublet at 2.90 ppm (2H) was assigned to the benzylic methylene and a quartet at 3.58 ppm was due to methine proton. The deuterium exchangeable signal at 2.3 ppm belongs to the two hydroxyl protons. The singlet at 1.3 ppm (6H, 2x-CH_3) integrating 6H corresponds to the gem-methyl of chromene moiety. The shapes, positions and proton content described as above was pin point the side chain undergo ring closure giving rise furan moiety form furanocoumarin occurring in many natural products from plants of umbellifereae family. Based on the above spectral evidences the structure of the compound assigned as furano coumarin rutaretin.[9][69-70] Finally, the structure of the compound had been confirmed by single crystal X-ray diffraction study as methyl ester of it.[71]

Fraction 12 was eluted with 25% MeOH in chloroform and volume of collected aliquot was 250 ml. This aliquot was evaporated using rota vapor to afford a dark brown sticky mass. It was gel permeated over sephadexLH20 previously saturated with 20% MeOH in chloroform. Five fractions were collected consisting approximate volume of each aliquot 50 ml. Combined fraction of first and second was consisting polymeric substance was discarded and residues obtained from other fractions were negligible analysis of these was incomplete on account of very low yield. The major constituent eluted in third fraction was a mixture of three components, purified by preparative TLC (15% MeOH in chloroform) as developing solvent system to separate out three bands coded as G-1, G-2 and G-3. These individual bands are scraped and eluted with suitable solvent(s). A pale-yellow amorphous powder was obtained to remove the solvent from band G-2, (R_f ~0.5) shown (+) ve Molisch test, coloration with neutral ferric chloride, typical UV absorption and smooth acid hydrolysis of it, resulted aglycon and sugar indicated that most probably it may be a O-glycoside-coumarin containing phenolic-OH group(s).[47] The sugar was identified as β-D (+) glucose by analyzing GCMS of its acetate and compared with respect to authentic sample. The characterization of the aglycone was done using spectroscopic methods and identified as a furanocoumarin known as ruterin.[15] Finally, its structure had been confirmed by single crystal X-ray diffraction study as its mono methyl derivative of its aglycon.

Therefore, structure of the glycoside was assigned as 2, 3-dihydro-2-(1-hydroxy-1-methylethyl- 9-β-D (+)-glucopyronosyl-7H-furo[3,2-g] benzo-pyran-7-one also known as ruteratin.[15] Analysis of others minor compounds seem to be phenolic glycosides and their analysis are incomplete on account of low yield.[70-72]

Compound 1: Pale yellow prism shape crystal; mp 120 °C (lit. 119-120 °C); UV data ($CHCl_3$), λ_{max} (log ε) :197 (0.20), 237 (0.19), 292 (0.18), 337(0.2). IR data (KBr), ν_{max}: 2925.0, 2854.0, 1742.5, 1626.3, 1597.3, 1456.6, 1410.0, 1376.3, 1291.8, 1258.5, 1156.7, 1115.5, 1074.7, 1018.2, 848.2, 754.9. ^{1}H NMR ($CDCl_3$, 200 MHz): 7.57 (d, *J* = 9.4 Hz, 1H, H-4), 7.19 (d, *J* = 8.6 Hz, 1H, H-6), 6.87 (d, *J* = 10.2 Hz, 1H, H-9), 6.70 (d, *J* = 8.4 Hz, 1H, H-6), 6.21 (d, *J* = 9.6 Hz, 1H, H-3), 5.71 (d, *J* = 10.2 Hz, 1H, H-10). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 160.9 (C-2), 156.1 (C-7), 149.9 (C-8a), 143.9 (C-4), 130.6 (C-9), 127.7, 114.8 (C-10), 113.4 (C-3), 112.4 (C-8 & C-4a), 109.0 (C-5), 77.5 (C-11), 28.0 (C-12 & C-13). EIMS data (70 ev), *m/z* (%): 228 (100), 213 (73.2), 199 (8.3), 185 (77.4), 158 (12.6), 142 (6.2), 128 (61), 115 (22.2), 102 (10.2), 92 (21.5), 77 (11.3), 63 (19.7). Accessed crystal structure using site: https://doi.org/10.1107/S1600536806008221

Compound 2: Needle shape colorless crystal, mp 162-163 °C (lit. 161-162 °C). UV data (MeOH), λ_{max} (log ε): 245 (4.12), 289 (3.84), 367 (4.16) nm. ^{1}H NMR ($CDCl_3$): δ_H 6.33 (d, 1H, *J* = 10 Hz, H-3), 7.75 (d, 1H, *J* =10 Hz, H-4), 7.41 (s, 1H, H-8), 6.8 (m, 1H, H-a), 7.65 (d, 1H, H-5).

Compound 3 (5, 7-dimethoxy coumarin): Colorless crystalline compound. It was identified as 5, 7-methoxy coumarin: 8.20 (d, 1H, *J* = 9.8 Hz, H-3), 7.65 (d, 1H, *J* = 2.0 Hz), 6.90 (d, 1H, *J* = 2.0 Hz, H-8), 6.30 (d, 1H, *J* = 9.8 Hz, H-3), 4.20 (s, 3H, -OCH_3). UV spectral data (MeOH), λ_{max} (log ε): 246 (3.77), 255 (3.77), 326 (4.17).

Compound 4: Colorless needle shape crystal (10% ethyl acetate in hexane), mp 185-87 °C (lit. 186-187 °C). UV data (MeOH), λ_{max} (log ε): 243 (4.13), 250 (4.20), 263 (4.19), 312 (4.14). ^{1}H NMR ($CDCl_3$, 200 MHz): 8.14 (d, 1H, *J* = 2.2, H-4), 7.57 (d, 1H, *J* = 2.2 Hz, H-3′), 7.12 (s, 1H, H-8), 7.02 (m, 1H, H-2′), 6.25 (d, 1H, *J* = 10 Hz, H-3), 4.25 (s, 3H, -OCH_3). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 161.41 (C-2), 112.59 (C-3), 105.19 (C-4), 152.78 (C-5), 139.44 (C-6), 144.91 (C-7), 158.48 (C-8a), 93.89 (C-9), C-5a (106.46), 105.19 (C-4a), 60.18 (C-10). EIMS data (70 ev), *m/z* (%): 216 (52.9), 201 (46.6), 189 (45.4), 173 (100), 159 (5.4), 145 (83.6), 132 (10.9), 117 (5.8), 106 (6.2), 98 (5.6), 89 (275), 74 (6.6), 63 (13.0). UV spectral data ($CHCl_3$), λ_{max} (log ε): 221(4.40), 242 (sh), 248 (4.20), 258 (4.17), 267 (4.20), 308 (4.18). ^{1}H NMR ($CDCl_3$, 500 MHz): δ_H 7.60 (d, J = 10 H, 1H), 7.20 (d, *J* = 10 H, 1H), 6.87 (d, *J* = 10 H, 1H), 6.72 (d, *J* = 10 H, 1H), 6.22 (d, *J* = 10 H, 1H), 5.73 (d, *J* = 10 H, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ_C 161.1, 156.39, 150.17, 130.87, 127.88. 115.08, 113.64, 112.68, 109.37, 77.72, 28.22 (2xCH_3). doi: 10.1107/S2056989016011221.

Compound 5: Needle shape colorless crystal, mp 143 °C. UV data (MeOH), λ_{max} (log ε): 221 (4.16), 240 (4.14), 247 (4.16), 270 (4.27), 311 (4.12). IR data (KBr), γ_{max}: 2925.5, 2853.9, 1712.2, 1627.5, 1509.7, 1455.8, 1377.5, 1288.2, 1230.3, 1093.3, 1046.8, 963.8, 746.0. ^{1}H NMR ($CDCl_3$, 200 MHz): δ_H 8.11 (d, *J* = 9.6 *Hz*, 1H, H-4), 7.56 (d, *J* = 2.2 Hz, 1H, H-3′), 7.00 (d, *J* = 2.2 Hz, 1H, H-2′), 6.23 (d, *J* = 9.6 Hz, 1H, H-3), 4.15 (s, 3H, -OCH_3), 4.04 (s, 3H, -OCH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 161.03, 158.10, 10, 152.41, 149.23, 144.5, 39.1, 112.2, 106.0, 104.9, 93.5, 60.1, 60.3. UV data ($CHCl_3$), λ_{max} (log ε): 245.5 (1.16), 265.5 (1.052), 311.0 (0.96). EIMS data (70 ev), *m/z* (%): [M^+] 246 (49.3), 231 (100), 204

(15), 188 (19.0), 175 (19.0), 160 (16.3), 147 (10.5), 104 (6.0).

Compound 6: 7-hydroxy-5-methoxy coumarin Colorless crystalline compound. It was identified as 5, 7-methoxy coumarin: 8.20 (d, 1H, *J* = 9.8 Hz, H-3), 7.65 (d, 1H, *J* = 2.0 Hz), 6.90 (d, 1H, *J* = 2.0 Hz, H-8), 6.30 (d, 1H, *J* = 9.8 Hz, H-3), 4.20 (s, 3H, -OCH_3). UV spectral data (MeOH), λ_{max} (log ε): 246 (3.77), 255 (3.77), 326 (4.17).

Compound 7: Colorless crystalline substance (10% ethyl acetate in hexane); mp 145-146 °C (lit. 147 °C). UV spectral data (MeOH), λ_{max} (log ε): 221 (4.12), 242 (3.84), 247 (3.84), 247 (sh), 260 (sh) 297 (4.16). ^1H NMR ($CDCl_3$, 200 MHz): δ_H 7.72 (d, 1H, *J* = 10 Hz, H-4), 7.65 (d, 1H, *J* = 2.3, H-3′), 6.78 (d, 1H, *J* = 2.3 Hz, H-2′), 7.31 (s, 1H, H-5), 6.32 (d, 1H, *J* = 10 Hz, H-3), 3.25 (s, 3H, 1x-OCH_3). EIMS data (70 ev), *m/z* (%): 216 (100) [M^+], 201(30.2), 189 (10.4), 173 (43.5), 145 (23.4), 89 (12.2).

Compound 8: Colorless crystalline solid, mp 275-276 °C (lit. 275-278 °C). ^1H NMR ($CDCl_3$, 500 MHz): 8.15 (d, 1H, *J* = 9.8 Hz, H-3), 7.59 (d, 1H, *J* = 2.0 Hz, H-3′), 7.13 (s, 1H, H-8), 7.02 (d, 1H, *J* = 2.0 Hz, H-2′), 6.27 (d, 1H, *J* = 9.8 Hz, H-3). UV data (MeOH), λ_{max} (log ε): 243 (4.13), 250 (4.20), 263 (4.19), 269 (4.27), 312 (4.14). EIMS data (70 ev), *m/z* (%): 208 (100), 189 (37.6), 165 (12.5), 149 (18.8), 137 (28.9), 122 (12.7), 109 (47.8), 95 (30.1), 81 (24.5), 67 (11.0), 55 (20.8).

Compound 9: Pale yellow crystalline solid; mp 147-148 °C; molecular formulae $C_{14}H_{16}O_6$ and mol. wt. 265 measured by EIMS. UV data (MeOH), λ_{max} (log ε): 220 (2.99), 266 (1.01), 333 (2.09). ^1H NMR ($CDCl_3$, 200 MHz): δ_H 7.52 (d, *J* = 9.6 Hz, 1H, H-4), 6.90 (s, 1H, H-8), 6.14 (d, *J* = 9.6 Hz, 1H, H-3), 4.74 (dd, *J* = 9.0 & 8.6 Hz, 1H, H-2′), 4.01 (s, 3H, -OCH_3), 3.20 (dd, *J* = 8.4 & 9.2 Hz, 1H, H-1′), 2.11 (brs, 1H, -OH) 1.35 (s, 3H, -CH_3), 1.22 (s, 3H, -CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 160.8 (C-2), 153.9 (C-5 & C-7), 147.2 (C-8a), 131.1 (C-4), 125.9 (C-3), 113.3 (C-4a), 112.2 (C-6), 91.4 (C-8), 71.5 (C-2′), 60.8 (C-3′), 29.8 (C-4′ & 5′). EIMS (70 ev), *m/z* (%): 265 (81.02), 246 (29.20), 229 (26.98), 204 (100), 191 (15.23), 176 (10.88), 146 (23.04), 127 (12.33), 96 (3.76), 88 (6.11), 71 (4.95), 60 (7.90).

Compound 10: ($CDCl_3$, 200 MHz): δ_H 7.60 (d, *J* = 9.6 Hz, 1H, H-4), 6.85 (s, 1H, H-8), 6.20 (d, *J* = 9.6 Hz, 1H, H-3), 5.02 (brs, 2H, 2x-OH), 5.35 (dd, *J* = 9.0, 8.6 Hz, 1H, H-6), 5.2 (s, 1H, 1x-OH), 4.96 (s, 1H, 1x-OH), 3.40 (dd, *J* = 8.4, 9.2 Hz, 1H, H-7a), 3.10 (dd, *J* = 8.0, 8.4 Hz, 1H, H-7b) 1.3 (s, 3H, 1x-CH_3), 1.20 (s, 3H, 1x-CH_3). EIMS data, *m/z* (%): 262 (M^+ not appeared), 224 (24.3), 229 (100), 201 (19.0), 198 (29.3), 187 (43.1), 155 (10.0), 131 (20.6), 115 (17.0), 100 (27.0), 89 (6.4), 75 (22.0), 63 (22.2), 51 (17.1), 43 (93.4). it is identified as 2′-hydroxy xanthyletin.

Compound 11: Colorless crystal (10% ethyl acetate in hexane); mp 147-148 °C (lit. 148-149 °C); UV data (MeOH), λ_{max} (log ε): 242 (4.16), 248 (4.16), 269 (4.27), 273 (sh, 4.27), 312 (4.12). ^1H NMR ($CDCl_{3,}$ 200 MHz): 8.08 (d, 1H, *J* = 10 Hz, H-4), 7.59 (d, 1H, *J* = 2.2 Hz, H-3′), 6.69 (d, 1H, *J* = 2.2 Hz, H-2′), 6.25 (d, 1H, *J* = 10 Hz, H-3), 4.14 (s, 6H, 2x-OCH_3). EIMS data (70 ev), *m/z* (%): [M^+] 246 (49.3), 231 (100), 204 (15), 188 (19.0), 175 (19.0), 160 (16.3), 147 (10.5), 104 (6.0).

Compound 12: Colorless crystal, mp 186-188 °C; molecular formulae $C_{14}H_{14}O_5$: mol. wt. 262 Da. (% yield 0.075 mg/gm). UV data ($CHCl_3$), λ_{max} (log ε): 237 (4.10), 292 (3.85), 337.0 (4.17) nm. IR data (KBr), ν_{max}: 3380.0, 3425.0, 1700.0, 1600 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ_H 7.65 (d, *J* = 9.5 Hz, 1H, H-4), 7.30 (d, *J* = 8.6 Hz, 1H, H-6), 6.24 (d, *J* = 9.5 Hz, 1H, H -3), 4.97 (d, 1H, *J* = 6.8 Hz, H-8), 3.83 (d, *J* = 6.8 Hz, 1H, H-8), 1.70 (brs,1H, -OH, D_2O exchangeable), 1.51 (s, 3H, -CH_3), 1.30 (s, 3H, -CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): Not recorded on account of its low yield. EIMS data (70 eV), *m/z* (%): 262 (16.7), 236 (100), 225 (13.1), 211 (28), 189 (4.0), 170 (15.7), 148 (19.4), 136 (34.3), 123 (38.9), 110 (30.0), 95 (95.3), 80 (88.7), 67 (12.12).

Compound (13 /5, 7-dihydroxy 6-prenyl coumarin): Light pale-yellow amorphous powder (yield 0.01%); mp: 185-187 °C; UV data (MeOH), λ_{max} (log ε): 252 (3.84), 256 (3.84), 324 (4.17). ^1H NMR ($CDCl_3$, 200 MHz): δ_H 7.62 (d, *J* = 9.8 Hz, 1H, H-4), 6.73 (s, 1H, H-8), 6.05 (d, *J* = 9.8 Hz, 1H, H-3), 5.02 (brs, 2H, 2 x-OH), 4.70 (dd, H-2′, *J* = 9.0 & 8.6 Hz, 1H,), 3.78 (s, 1H,1x-OH), 3.15 (d, 2H, *J* = 8.8 Hz), 1.3 (s, 3H, 1x-CH_3), 1.20 (s, 3H, 1x-CH_3). ^{13}C ($CDCl_3$, 50 MHz): δ_C 162.1 (C-2), 151.0 (C-8a), 145.2 (C-5), 143.2 (C-7), 128.2 (C-4a & C-8), 125.7 (C-3′), 113.2 (C-2′), 110.5 (C-8), 47.7 (C-1′), 29.7 (C-5′), 24.0 (C-4′). IR data (KBr), ν_{max}: 3163.4, 3126.6, 2920.50, 2854.3, 1752.9, 1720.6, 1613.0, 1591.6, 1480.5, 1430.3, 1355.0, 1208.0, 1143.4, 1079.0, 1010.9, 950, 817.4, 754.4. EIMS data (70 ev), *m/z* (%): 246 (98.2), 232 (100), 204 (22.2), 188 (37.1), 175 (37.5), 160 (27.4), 147 (14.8), 131 (7.4), 119 (11.5), 104 (11.5), 87 (11.1), 76 (12.0), 66 (6.9).

Compound 14: It has been identified as oroselone by single crystal X-ray diffraction study accessed by using site https://doi.org/10.1107/S2056989016003303

Compound 15: Hygroscopic substance; mp uncorrected; molecular formulae $C_{20}H_{26}O_{11}$ and molecular weight 442 determined by HRMS. UV data (MeOH), UV spectral data (MeOH), λ_{max} (log ε): 220 (2.99), 266 (1.01), 333 (2.09). IR data (KBr), ν_{max}: 3554.5, 3012.62, 2986.40, 2907.7, 1666.7, 1614.6, 1579.6, 1492.3, 1431.1, 1387.4, 1308.8, 1247.6, 1160.0, 1081.4, 1011.7, 959.3, 793.2, 749.5. ^1H NMR ($CDCl_3$, 200 MHz): δ_H 7.75 (d, *J* = 9.4 Hz, 1H, H-4), 6.93 (s, 1H, H-8), 6.15 (d, *J* = 9.4 Hz, 1H, H-3), 4.76 (d, *J* = 4.6 Hz, 1H, anomeric H of sugar), 3.78-3.13 (complex multiplet, 12H, sugar protons), 1.34 (s, 3H, 1x-CH_3), 1.30 (s, 3H, 1x-CH_3). ^{13}C NMR ($CDCl_3$, 50 MHz): δ_C 160.1 (C-1), 155.0 (C-5 & C-8a), 145.3 (C-5), 143.4 (C-7), 128.3 (C-4), 125.8 (C-6), 114.0 (C-3), 113.9 (C-4a), 110 (anomeric-C), 77.9 (C-4″), 76.6 (C-3″), 76.0 (C-2″), 73.5 (C-1″), 69.9 (C-6″). EIMS data, *m/z* (%): 444 [M^+-not appeared], 263 (83.2), 245 (38.6), 229 (26.9), 176 (10.9), 204 (100), 174 (3.1), 127 (2.3), 97 (3.8), 85 (5.3), 71 (3.5).

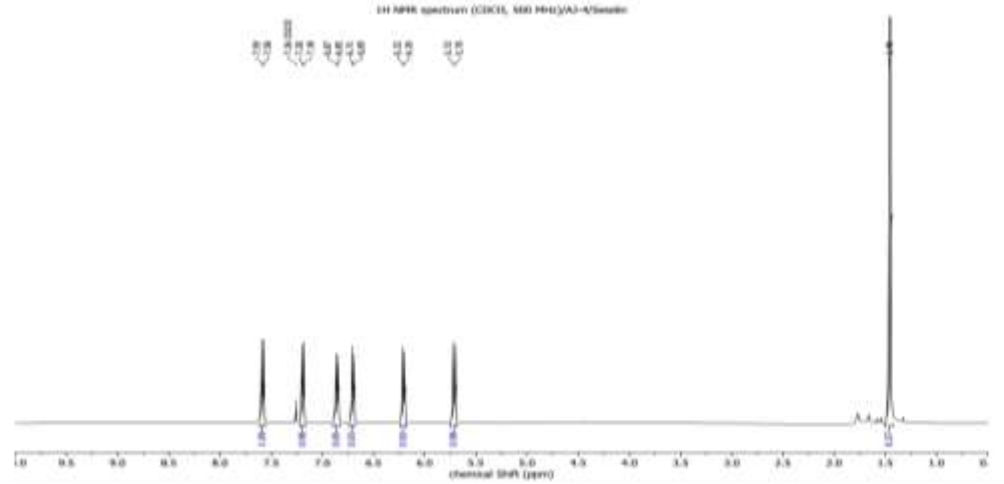

Figure S1: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (Seselin).

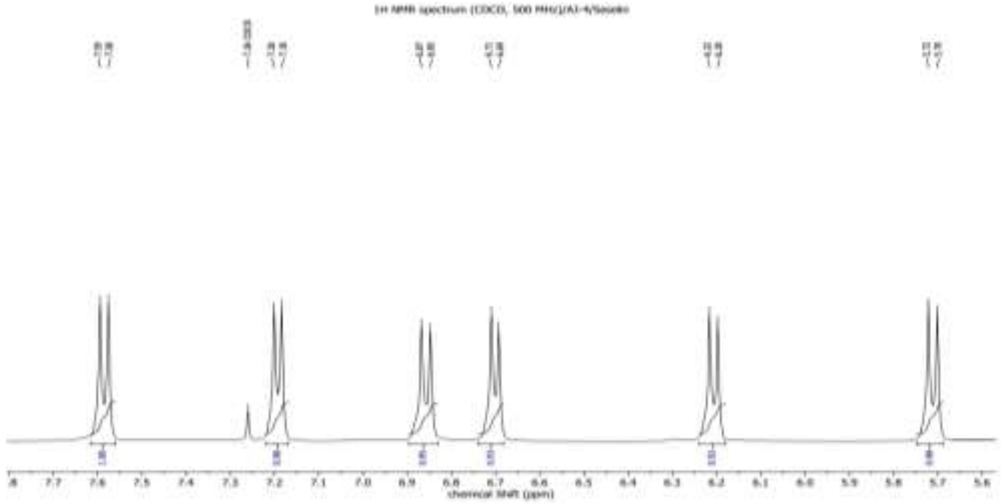

Figure S2: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (Seselin).

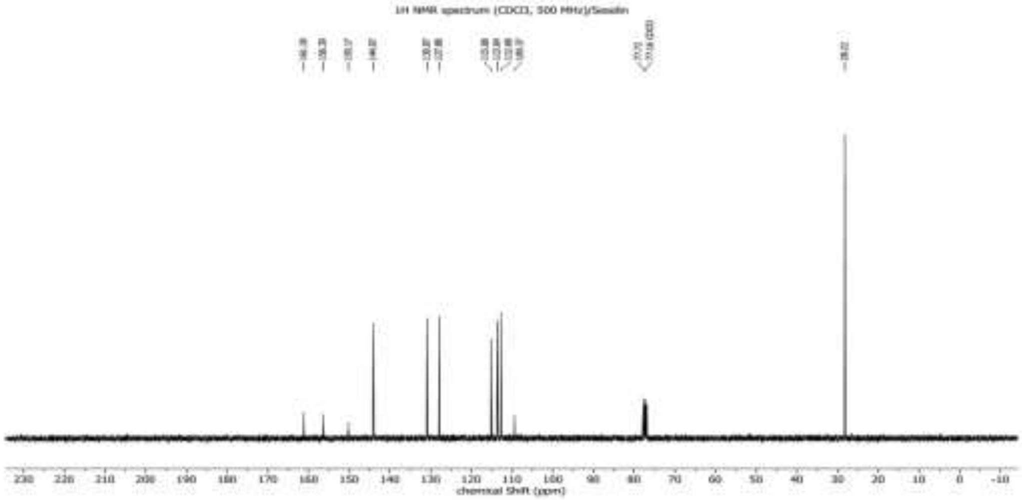

Figure S3: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 1 (Seselin).

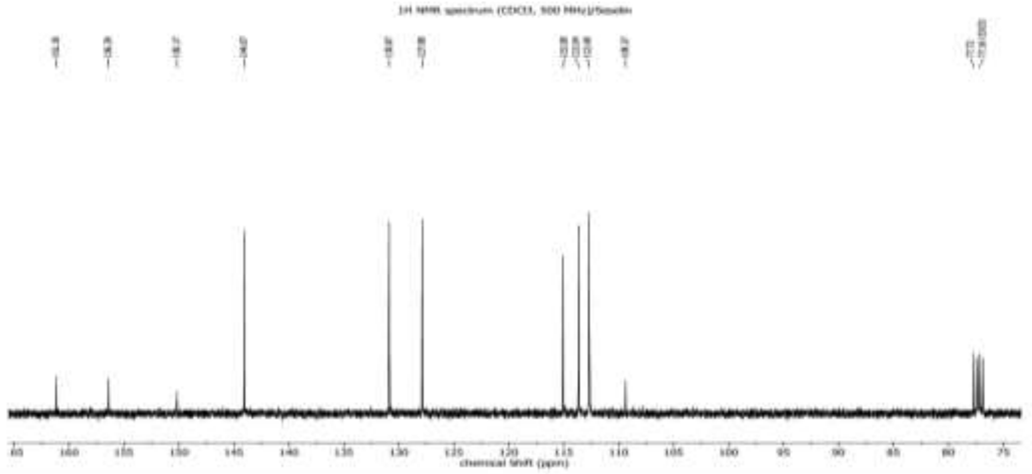

Figure S4: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 1 (Seselin).

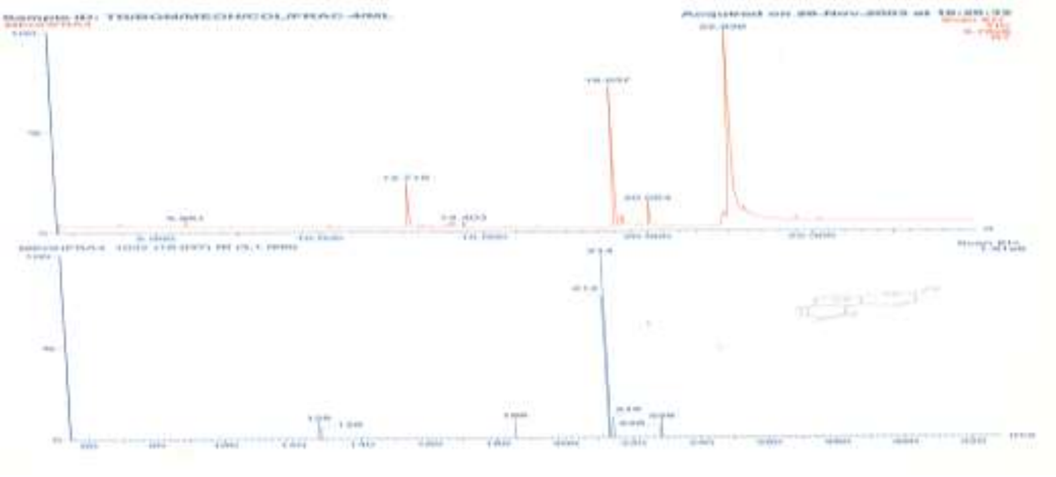

Figure S5: EIMS-spectrum of compound 1 (Seselin).

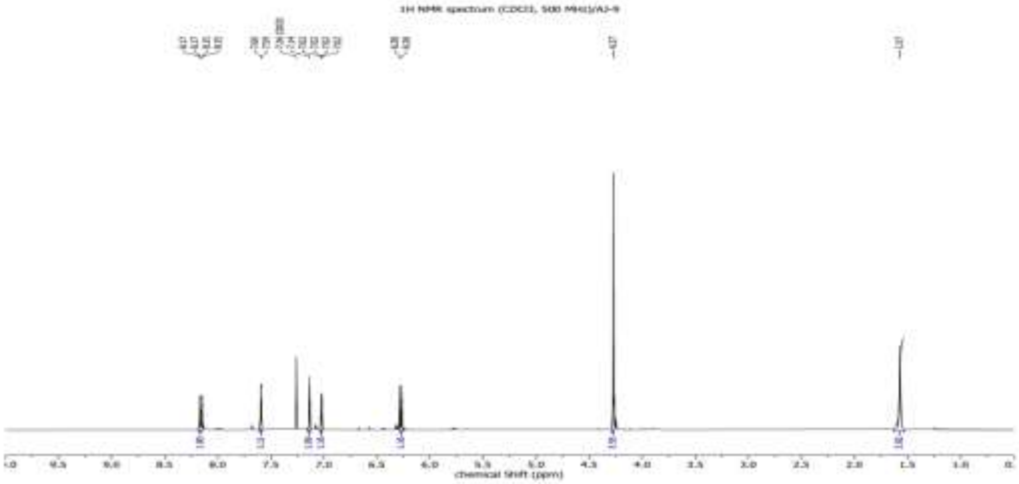

Figure S6: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 4 (Bergapten).

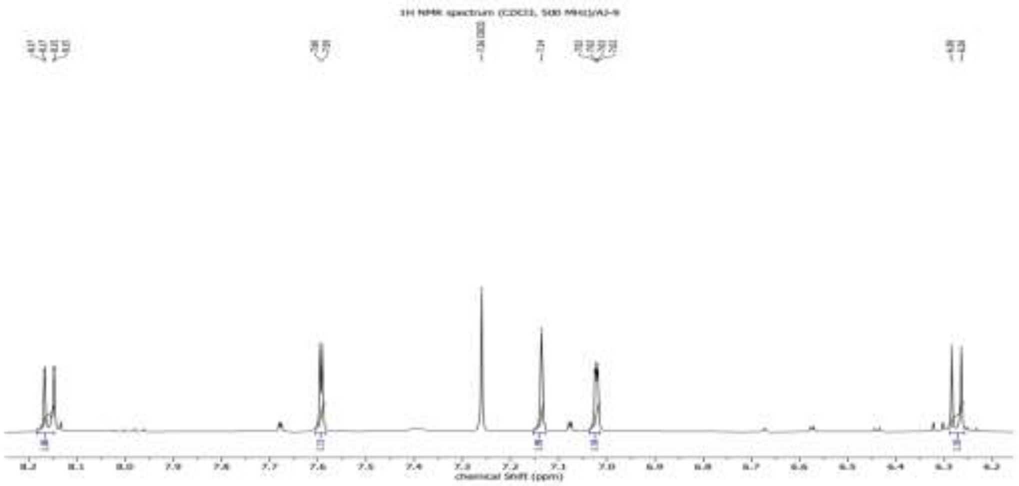

Figure S7: Expansion of ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of compound 4 (Bergapten).

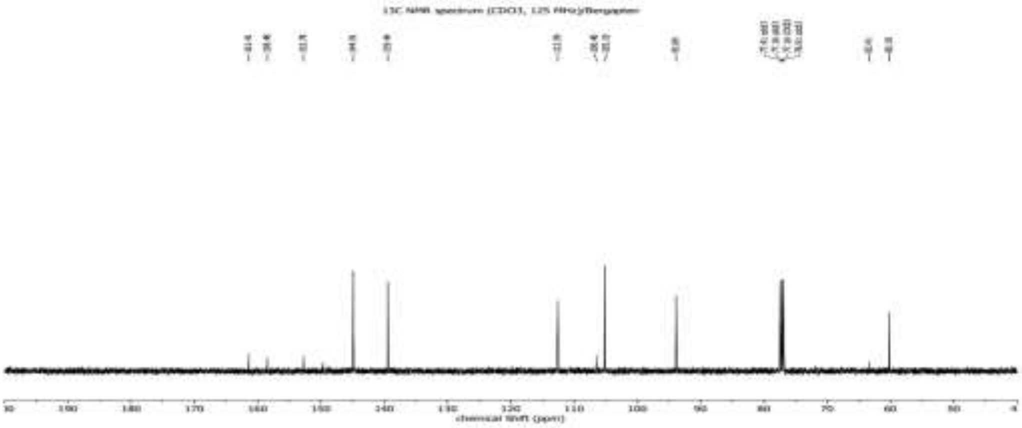

Figure S8: ^{13}C NMR spectrum (CDCl$_3$, 125 MHz) of compound 4 (Bergapten).

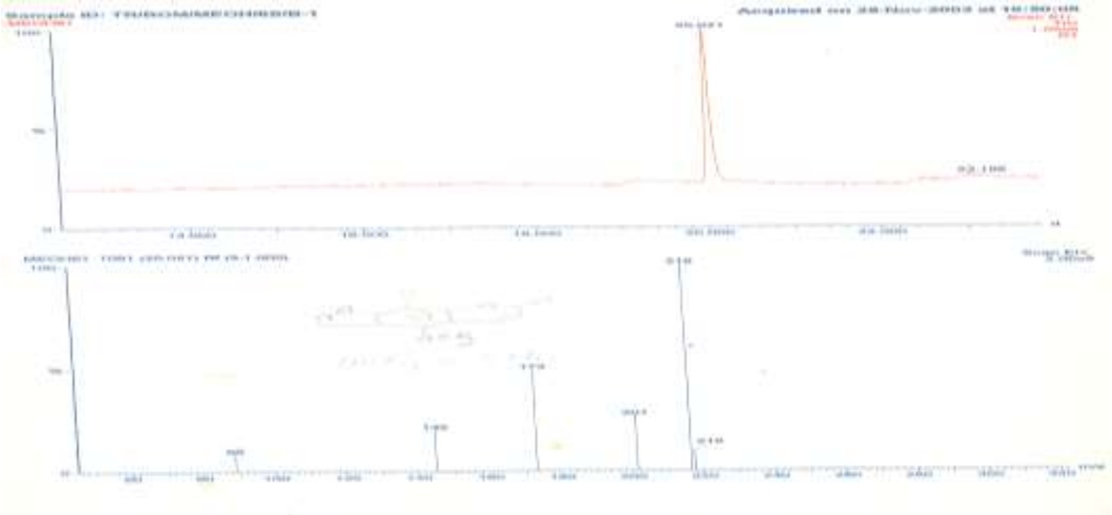

Figure S9: EIMS-spectrum of compound 4 (Bergapten).

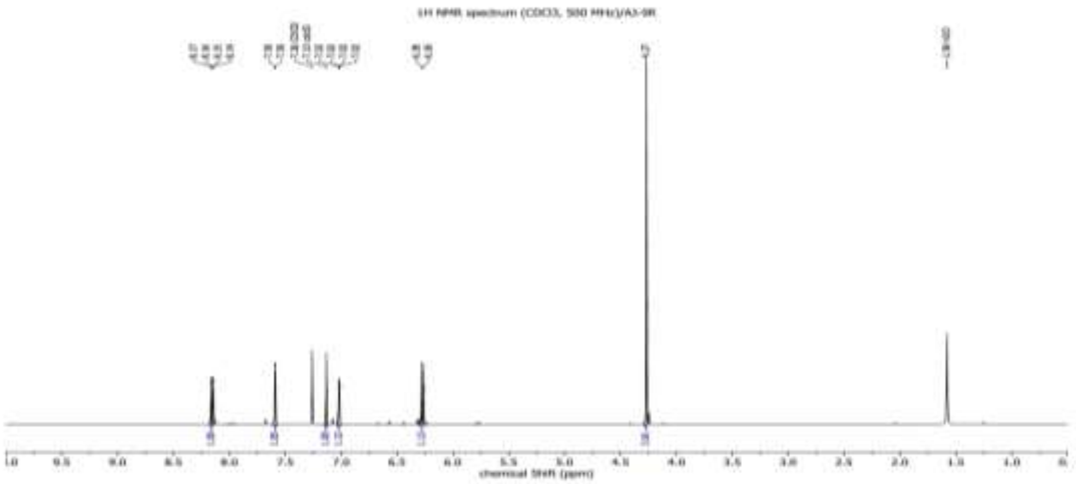

Figure S10: ^{1}**H NMR spectrum (CDCl$_3$, 500 MHz) of compound 7 (xanthotoxin).**

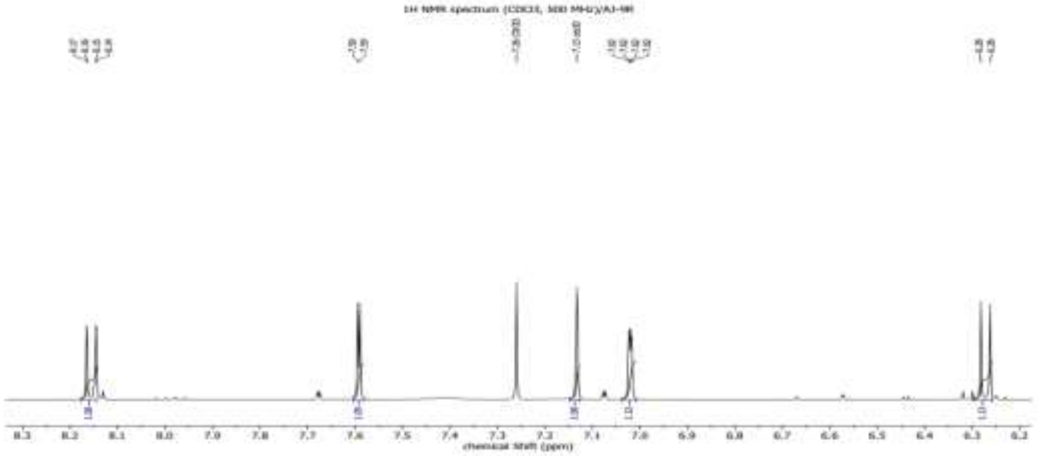

Figure S11: Expansion of ^{1}**H NMR spectrum (CDCl$_3$, 500 MHz) of compound 7 (xanthotoxin).**

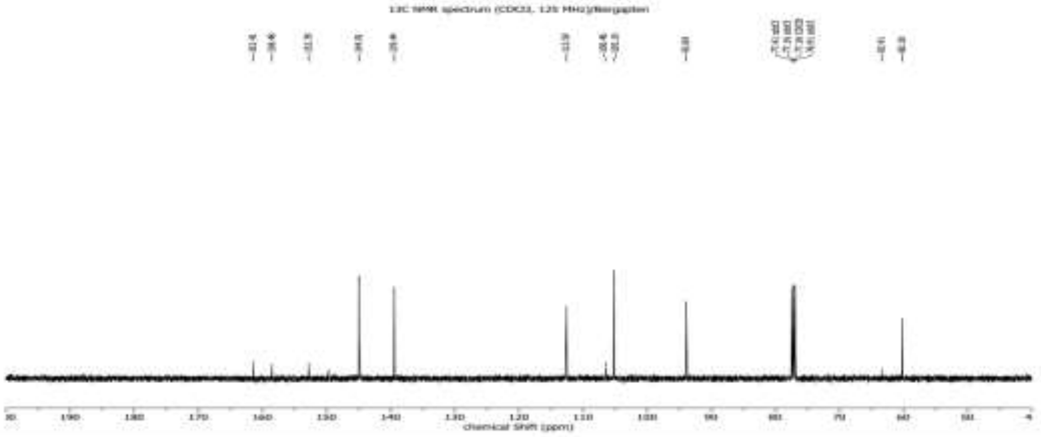

Figure S12: ^{13}**C NMR spectrum (CDCl$_3$, 125 MHz) of compound 7 (Xanthotoxin).**

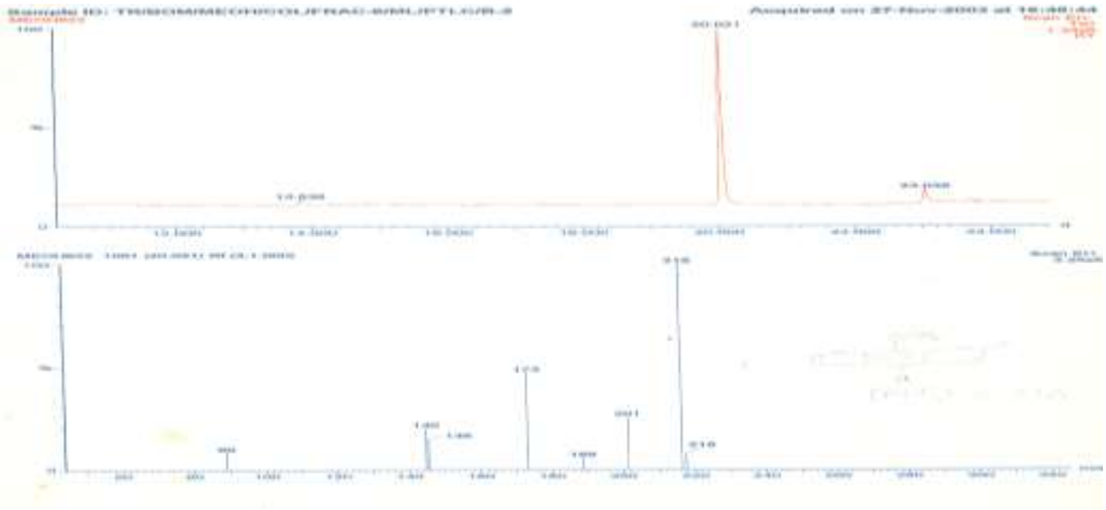

Figure S13: EIMS-spectrum of compound 7 (Xanthotoxin).

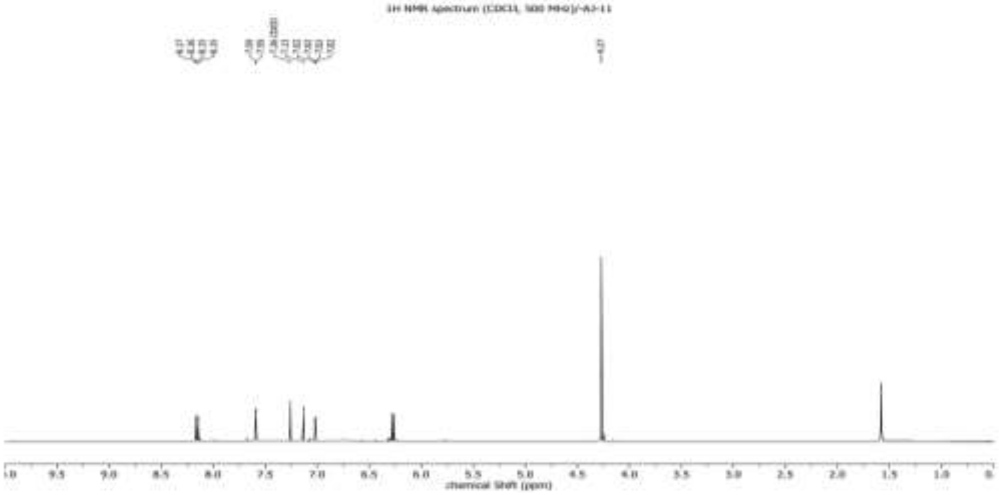

Figure S14: ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of methoxy derivative of compound 8 (bergaptol).

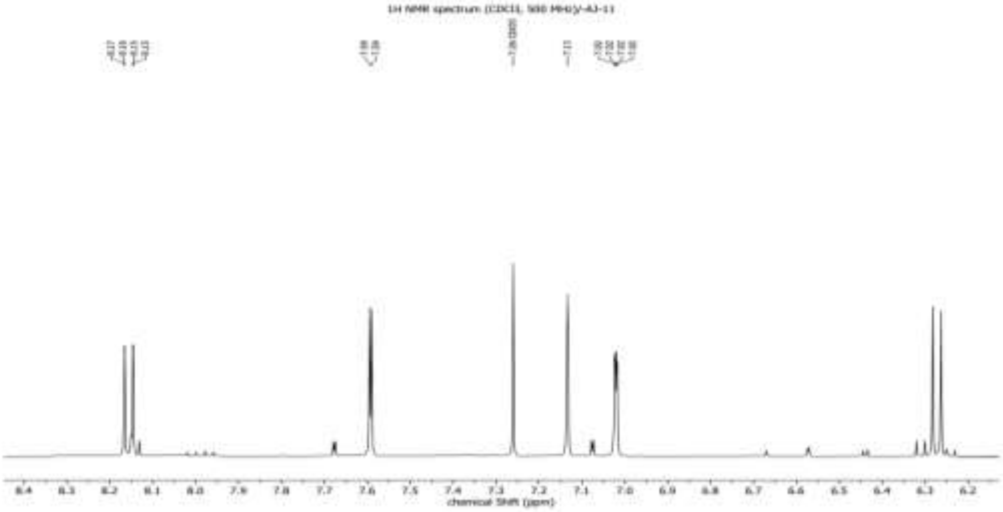

Figure S15: Expansion of ^{1}H NMR spectrum (CDCl$_3$, 500 MHz) of methoxy derivative of compound 8 (bergaptol).

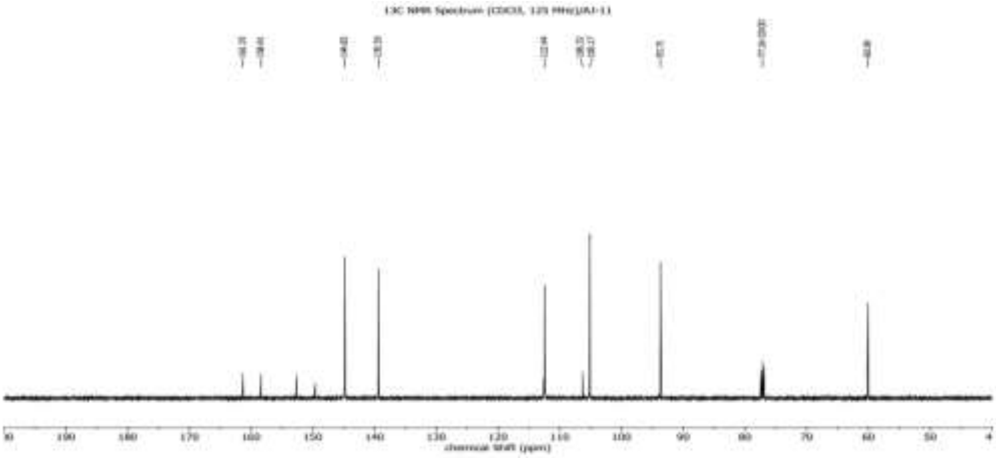

Figure S16: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of methoxy derivative of compound 8 (bergaptol).

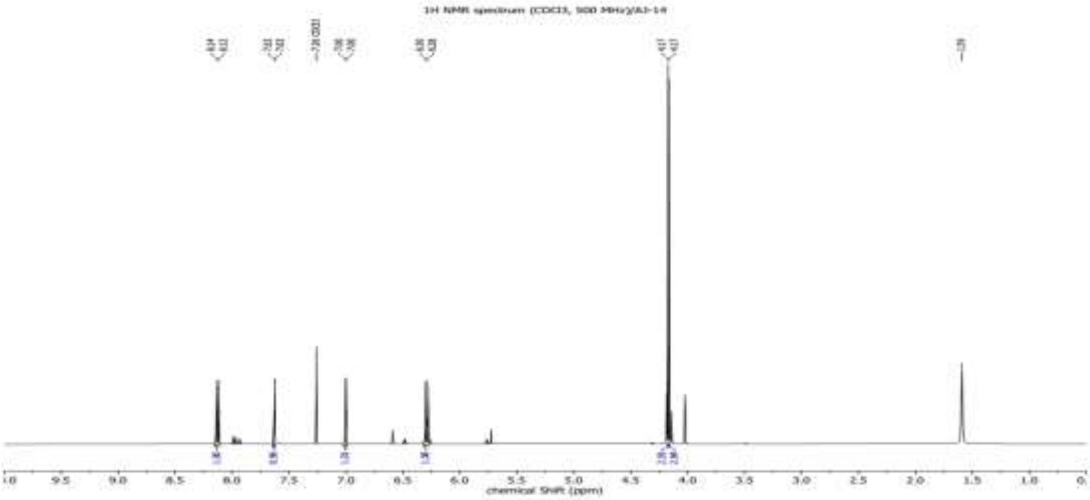

Figure S17: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 7 (Isopimpinellin).

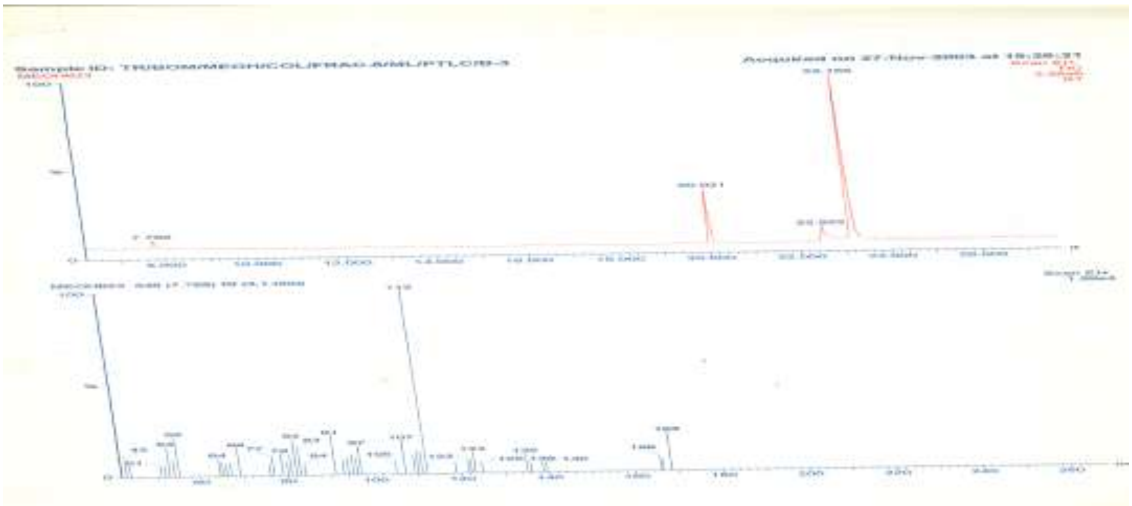

Figure S18: EISM spectrum of compound 7 (Isopimpinellin).

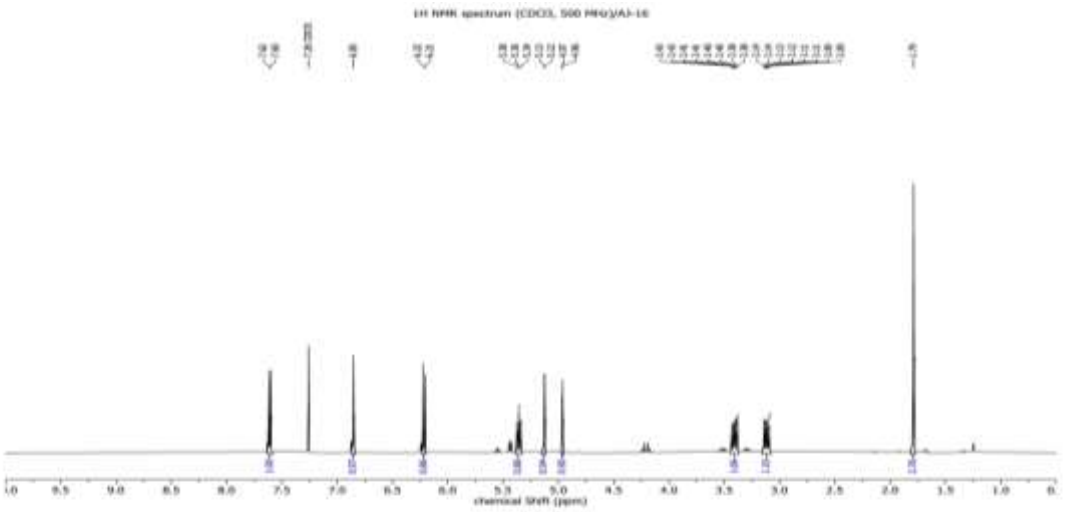

Figure S19: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 9 (Ruteratin).

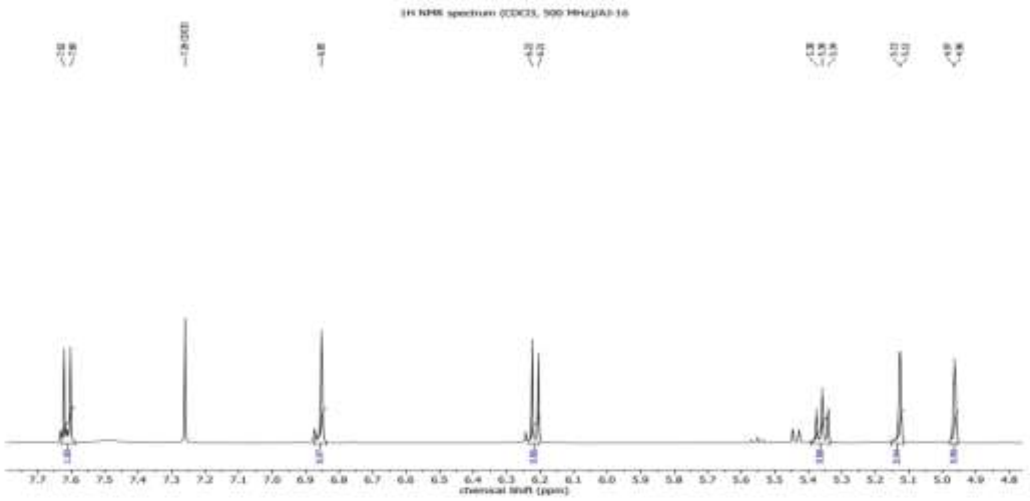

Figure S20: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 9 (Ruteratin).

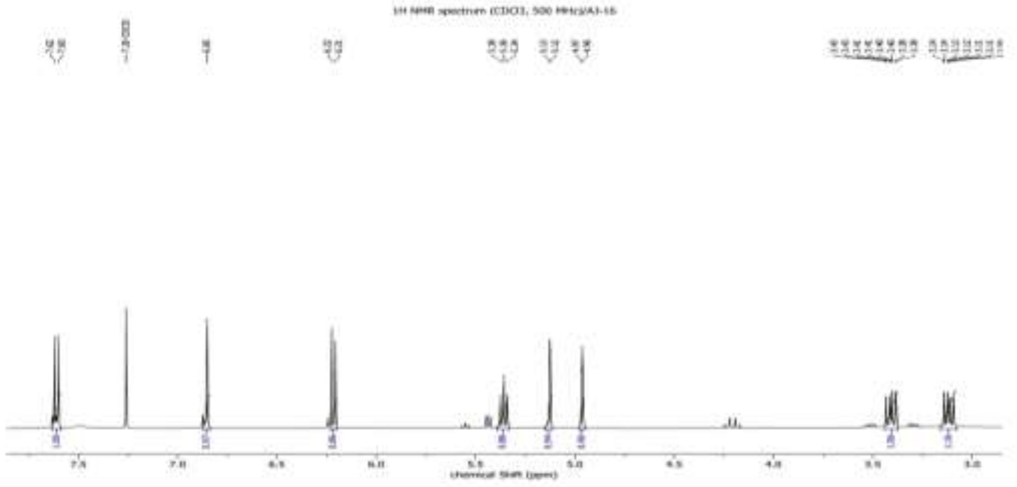

Figure S21: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 9 (Ruteratin).

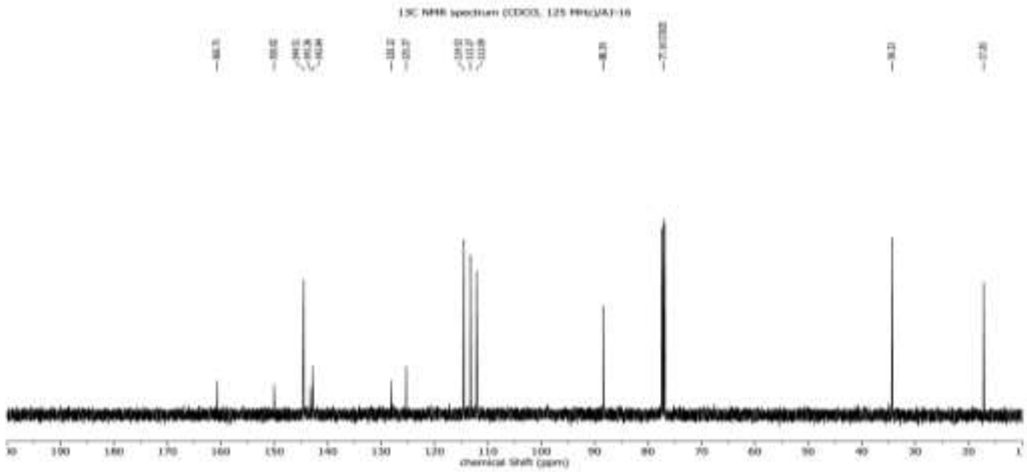

Figure S22: ^{13}C **NMR spectrum (**$CDCl_3$**, 125 MHz) of compound 9 (Ruteratin).**

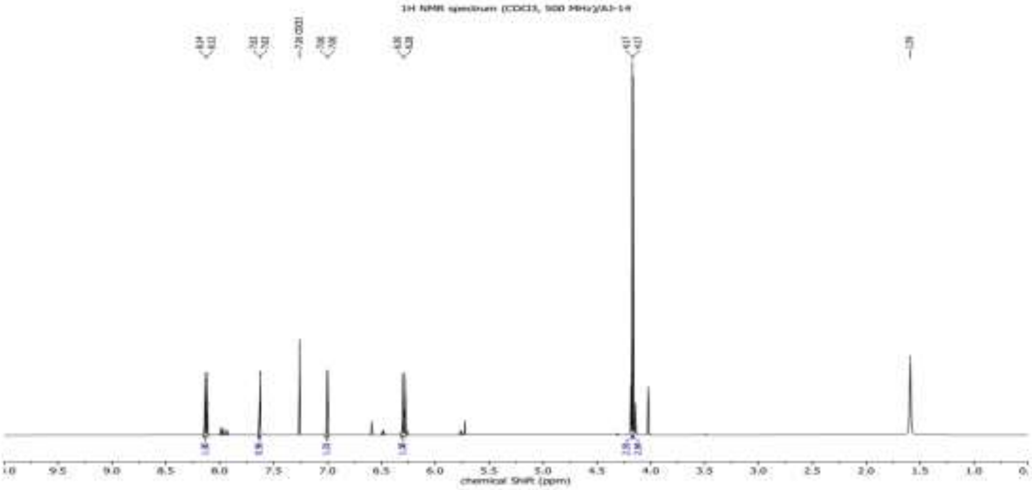

Figure S23: ^{1}H **NMR spectrum (**$CDCl_3$**, 500 MHz) of compound 7 (pimpinellin).**

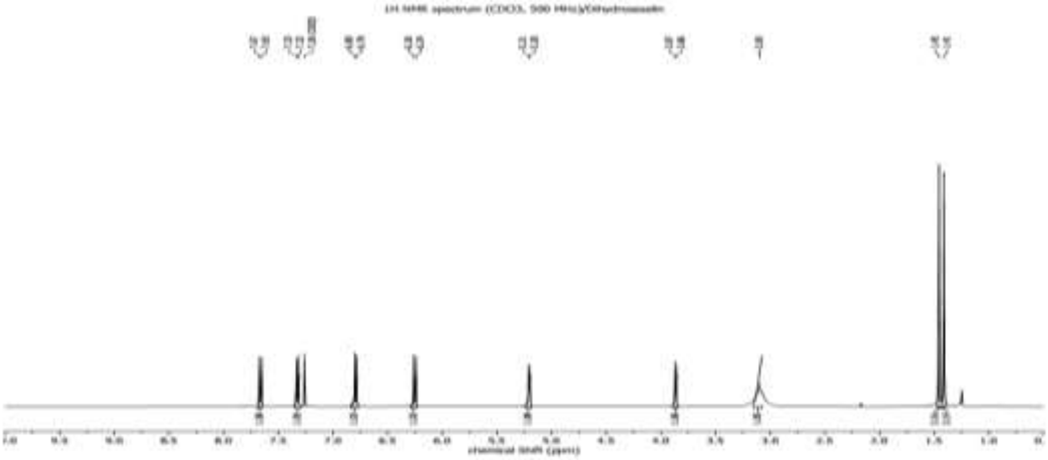

Figure S24: ^{1}H **NMR spectrum (**$CDCl_3$**, 500 MHz) of compound 12 (Dihydroseselin).**

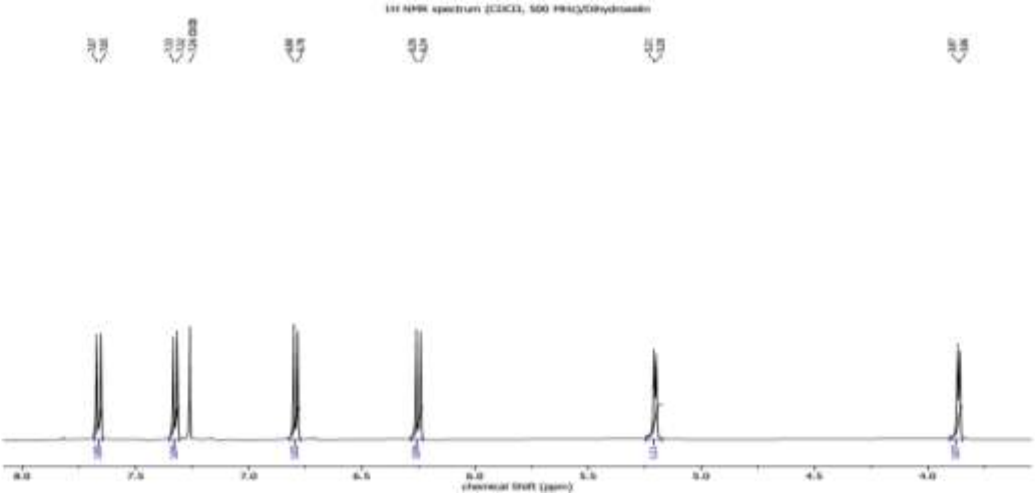

Figure S25: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 12 (Dihydroseselin).

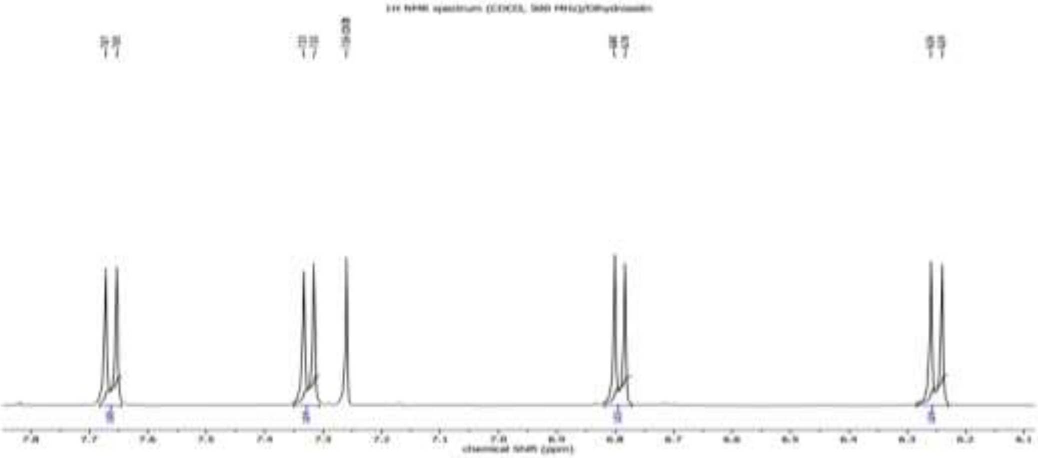

Figure S26: Expansion of 1H NMR spectrum ($CDCl_3$, 500 MHz) of compound 12 (Dihydroseselin).

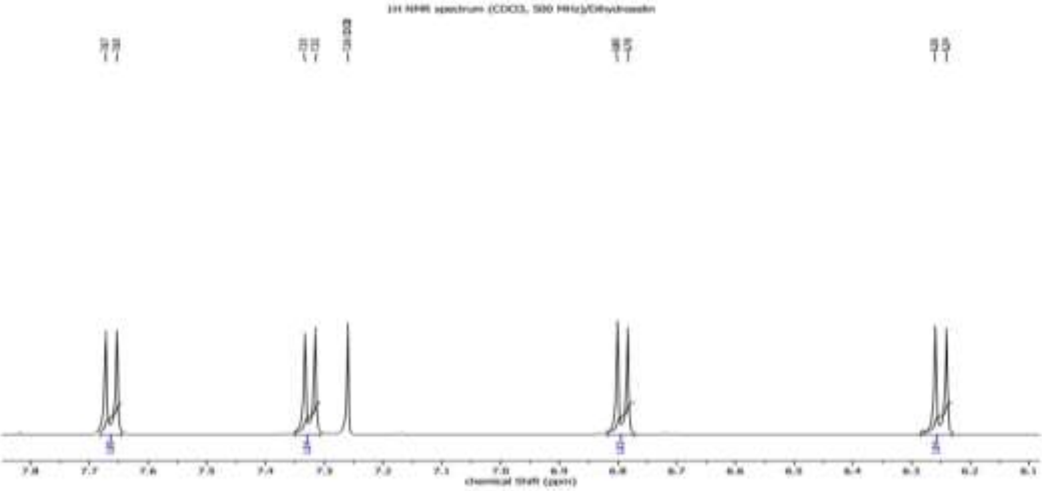

Figure S27: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 12 (Dihydroseselin).

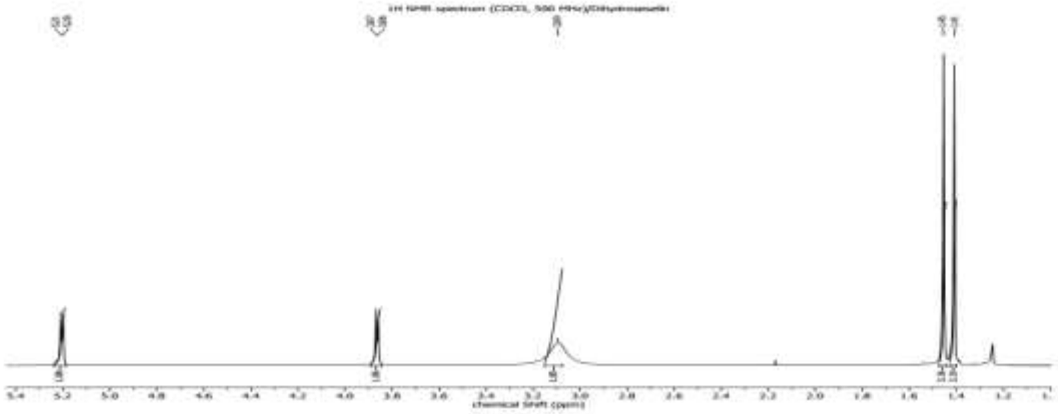

Figure S28: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 12 (Dihydroseselin).

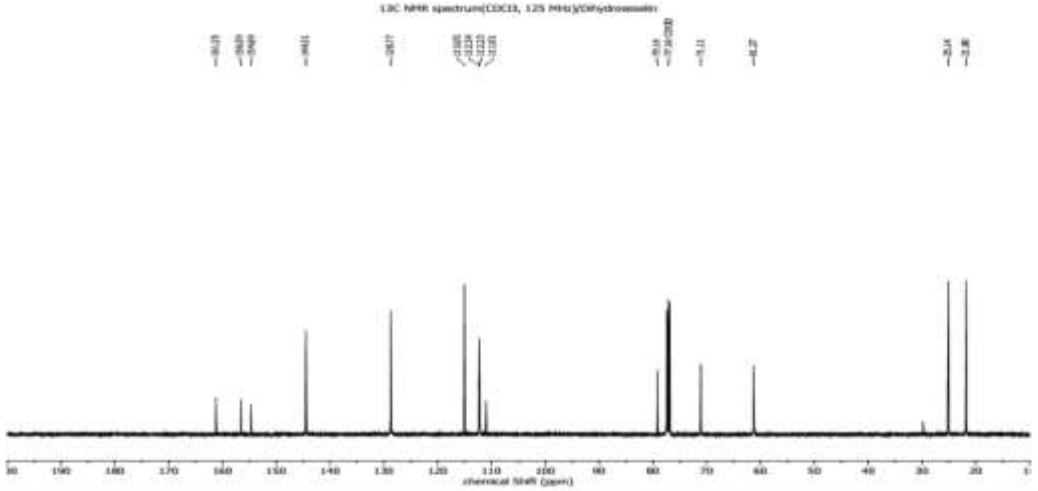

Figure S29: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 12 (Dihydroseselin).

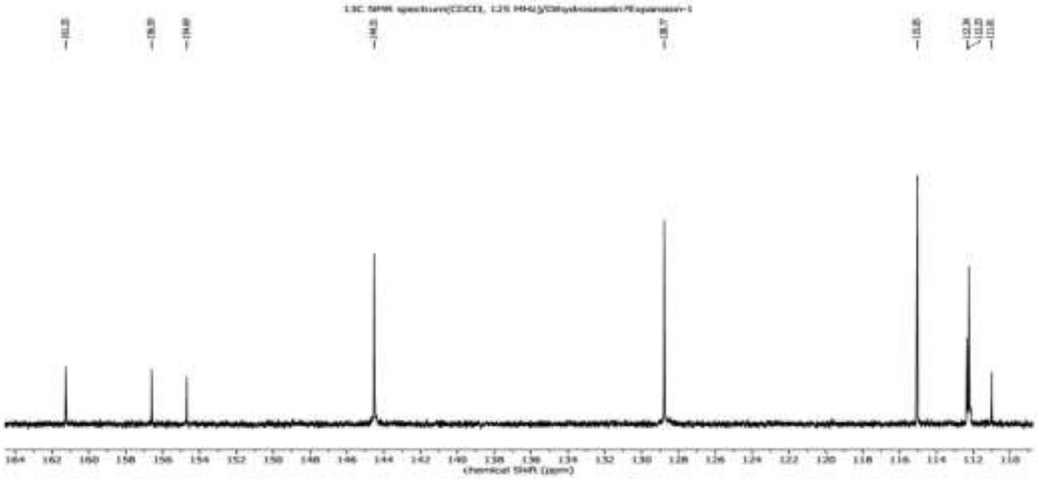

Figure S30: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 12 (Dihydroseselin).

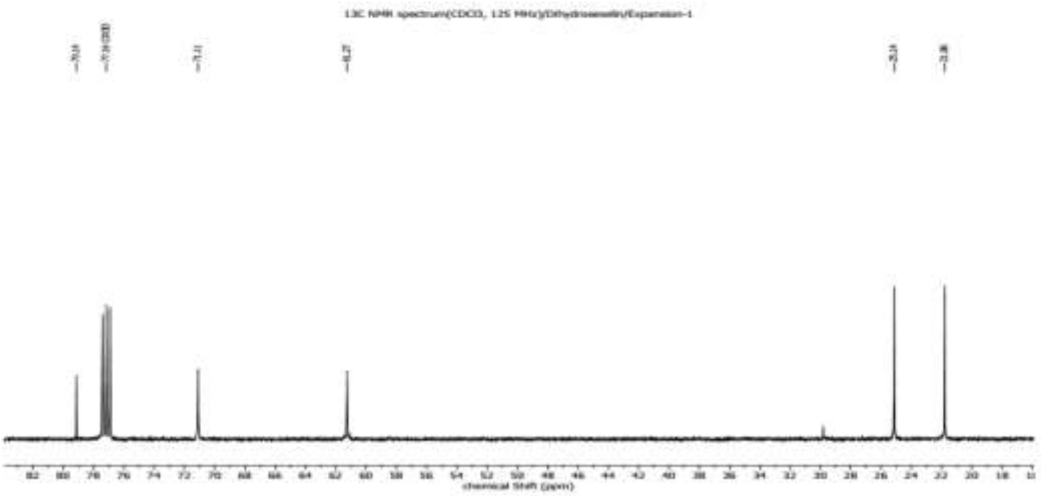

Figure S31: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 12 (Dihydroseselin).

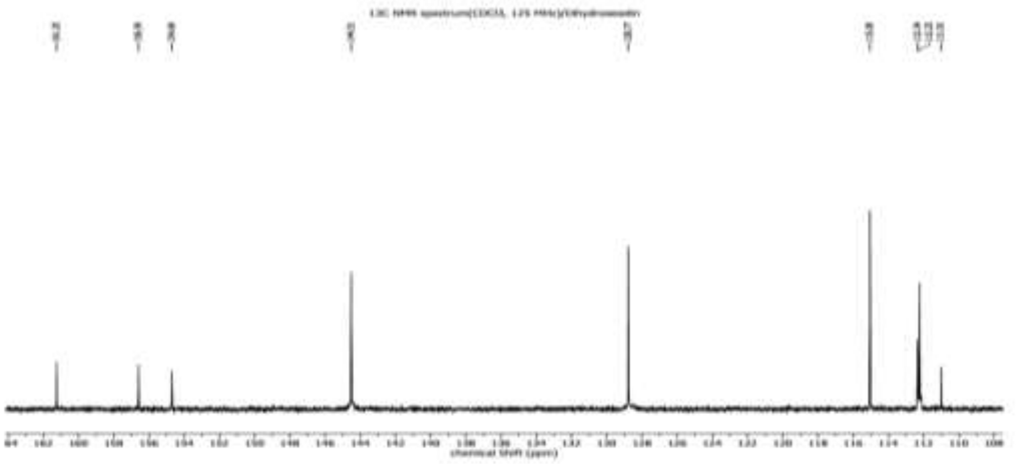

Figure S32: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 12 (Dihydroseselin).

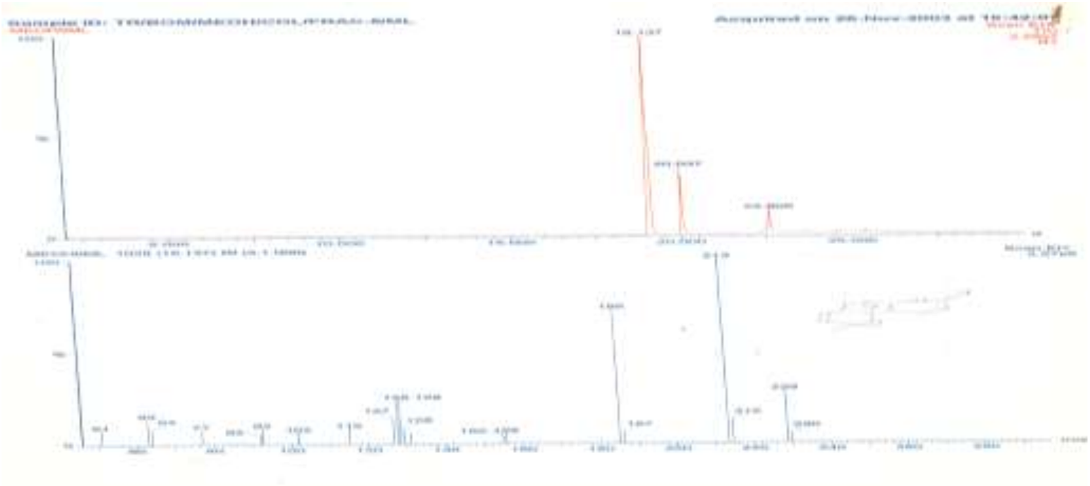

Figure S33: EISM spectrum of compound 12 (Seselin formed on dehydration while on mass).

CONCLUSION AND SUMMARY OF WORK

The seed of Trachyspermum stictocarpum (Ajmoda seed) is one of the most popular Indian spices for improving digestion strength and an iconic condiment in Indian cuisine. Several O-heterocyclic compounds belonging to coumarins has been isolated from its methanol extract by column chromatography with gradient solvent system. Their structural characterization has been carried out by means of spectroscopic and spectrometric methods. The structural confirmation of crystallized coumarin has been secured by single crystal X-ray diffraction study.

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Supplimentary information

Supplementary information for this paper is available in ANNEXURE 1.

ACKNOWLEDGEMENTS

Authors are very much grateful to Head of Division, Bio-Organic Division and Group Director of Bio-science Group for their continuous encouragement and ethical support.

Author contribution statement

AKB performed isolation, structural characterization, total experimental design, work and analyses and prepared manuscript. SF contributed analysis tools and analysed the XRD data, structure analysis and computation.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Additional information

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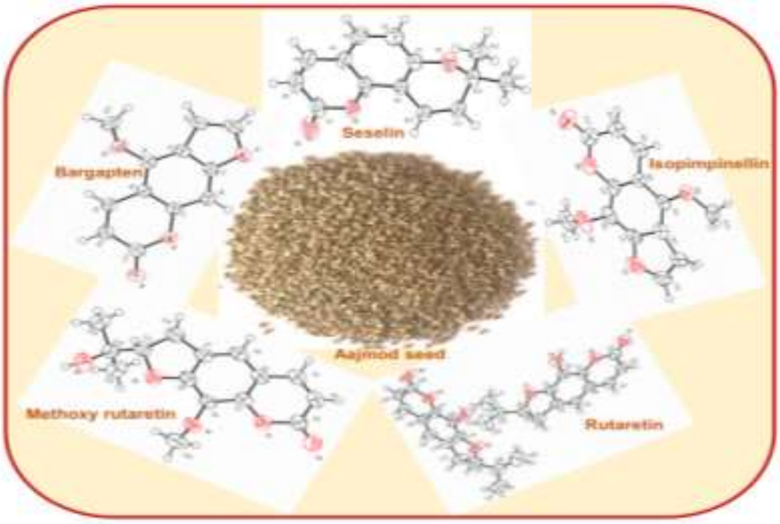
Competing financial interest

The author declares no competing financial interest.

Graphical abstract



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