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PHYTOCHEMICAL PROFILING AND CHROMATOGRAPHIC ANALYSIS OF ACETONE EXTRACT OF *BOERHAAVIA DIFFUSA* THROUGH THIN LAYER CHROMATOGRAPHY & GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Boerhaavia diffusa (punarnava), a member of the Nyctaginaceae family, is a well-known plant in India for its antioxidant and hepatoprotective activities. The present study was conducted to identify the novel compounds based on the novel medicinal properties of the plant. Preliminary phytochemical screening, TLC, and GC-MS-based chromatographic analysis of an acetone extract from *Boerhaavia diffusa.* Resulted in the detection of carbohydrates, alkaloids, saponins, tannins, polyphenols, and glycosides in the plants. Compounds were studied through TLC using solvents mixture as a mobile phase in the ratio chloroform: methanol: ammonia (2:2:1) at 254 and 365 nm.The GC-MS analysis of *Boerhaavia diffusa* resulted in detail of more than 200 compounds present in different peaks. All of them were cross-checked at various online compound databases such as Pubchem, and Drug bank for the identification of their biological activities, if already reported. Mainly 25 compounds (falling in 50.10% peak area) like 2-None-1-ol, (E)-, (Z)-, trans-2-Undecen-1-ol, 2-Propylcyclohexanol, Ethanol pentamethyl,Formamide, N-methyl- etc. were characterized in the study. According to the given medicinal properties of the identified compounds in compounds databases, the plant is hereby reported for possessing the Nematocidal, Anticancer, Antitumor, Antimicrobial, and Antioxidant Activities. The presence of various bioactive compounds justifies the use of the plant for various ailments. However, the Isolation of individual phytochemical components and their pharmacological evaluation will yield more fruitful results.

Keywords: *Boerhaavia diffusa,* GC-MS based study, Phytochemical Screening, Punarnava, Soxhlet extraction.

Introduction

Boerhaavia diffusa is a species of flowering plant belonging to the Nyctaginaceae (four o'clock) family[1]. It is commonly known as punarnava, (capable of body rejuvenation) and is a well-known plant in India for its antioxidant and hepatoprotective activities. It is taken as the main ingredient in many herbal medicines for pain relief and other purposes. Different parts of the plant are traditionally known for their several medicinal properties. Due to the frequent use of this plant against various diseases such as perfusion of the renal system, treatment of semen deficiency, blood pressure and as an appetite stimulant, alexiteria, intestinal colic, kidney diseases, cough, hemorrhoids, skin diseases, alcoholism, insomnia, eye diseases, asthma, and jaundice, it is considered as a plant of great medicinal value in Ayurveda[2,3]. It is used in the treatment of several diseases like cardiovascular and renal disorders[4,5], memory and cognitive function age-related neurological dysfunctions such as Alzheimer's disease[6,7,] ulcers[8], and several other human

ailments. The plant is widely distributed in India, Sri Lanka, Egypt, Sudan, Ghana, South Africa, Nigeria, China, Australia, Philippines, Iran, etc[9,10]. However, the genus *Boerhaavia* L. (Family: Nyctaginaceae) consists of 40 tropical and subtropical species that wildly occur in diverse terrestrial habitats ranging from managed grassland, wasteland, and agro ecosystems to large forest gaps.The medicinal value of the plant lies in the bioactive phytochemical components that produce certain physiological effects on the human and animal bodies. A phytochemical is a natural bioactive compound found in plant foods that works with nutrients and fiber to protect against disease.However, a considerable number of works have been done on the identification of phytochemical compounds in different parts of *Boerhaavia diffusa* which have reported the compounds possessing the anti-parasitic, antifungal, antibacterial, antiviral, antihistaminic, antioxidant, and hepatoprotective activities[11,12]. But no GC-MS-based chromatographic studies are reported on acetone extract from the leaves of the plant. Since, being a polar molecule extraction in Acetone solvent (polarity-0.355) may help in getting compounds extracted with matching polarity therefore in our study the leaves are extracted in Acetone solvents for studying the extracted phytocompounds through GC-MS and validating their medicinal properties reported at the compound databases such as Drug Bank, Pubchem, etc.

Material and methods

Collection of the plant material:

Boerhaavia diffusa leaves were collected in October and authenticated by the Central Council for Research in Ayurvedic Science (CCRAS) – Regional Ayurveda Research Institute Gwalior Road, Jhansi Uttar Pradesh in November 2019 (accession number 28569).

The collected fresh plants were first washed with tap water and distilled water. In addition, the leaves were shade dried for 2-3 weeks and converted to a fine powder using an electric mixer in front of the Soxhlet extraction.

Preparation of plant extract:

The crushed uniform powder (10 g) was placed in a Soxhlet apparatus with acetone (250 ml) as a solvent for extraction at 56°C for 16-18 hours. The collected pure extract from leaves of *Boerhaavia diffusa* was filtered with Whatman filter paper No. 41 (110 mm). After filtration, the extract was concentrated on a rotary evaporator. Model details: EYELA N-12008, EYELA-UNI TRAP UT-1000 with water bath set at 45-50C. The resulting solution was stored in a refrigerator at 4°C for further analysis.

Preliminary phytochemical screening:

Preliminary phytochemical screening and qualitative tests were performed using the standard methods for detecting carbohydrates, alkaloids, amino acids, phenol, tannins, Flavonoids, Saponins, terpenoids, quinines, cardiac glycosides, and steroids in the extract by characteristic color change.

Detection of Carbohydrates:

Molisch’s test: 2 ml of plant extract and 1 ml of Molisch's reagent and 1 ml of concentrated sulfuric acid were added through the sides of the test tube and then observed for the formation of a violet/purple ring[13].

Benedict's test: 2 ml of the extract was taken and a few drops of Benedict's reagent were added to the extract and boiled in a water bath for 2 minutes. It was allowed to cool down and then observed for the formation of a red, yellow, or green precipitate[13].

Detection of Alkaloids:

Mayer’s Test: The filtrate was treated with 1 ml Mayer’s reagents (potassium mercury iodine) and observed for the formation of a yellow color.

Detection of Saponins:

Foam test: 2 g powder extract was diluted in 20 ml distilled water and shaken in a graduated cylinder for 15 min and then observed for foam layer formation.

Detection of Polyphenols:
Ferric chloride test: About 2 ml of plant extract was mixed in 5 ml of distilled water and heated at 45-100°C for 5-10 minutes. Then 2 ml of 0.3% $FeCl_3$ was added to the mixture and observed for the formation of the green or blue color.
Detection of Tannins:
Ferric chloride test: A 0.5 g sample of powder was taken in 20 ml distilled water and boiled for 10 minutes, then filtered with Whatman filter paper (#41; 110 mm), and 2-3 drops of 0.1% $FeCl_3$ were added to the mixture and then observed for change of color of the solution to brownish-green-black or blue-black color.
Detection of cardiac glycosides:
Keller-Killani test: 5 ml extract was mixed with 2 ml. Glacial acetic acid and one drop of ferric chloride solution were added, followed by the addition of 1 mL of conc. H2SO4 Observed for the brown ring and the violet ring.
Detection of Flavonoids
NAOH test: 2 ml of the extract and a few drops of Sodium Hydroxide solution were placed in a test tube. After the formation of intense yellow color, 2-3 drops of dilute HCl were added to the mixture and observed for color change.
Detection of Quinines
KOH test: 2 ml of the extract was taken and 1 ml of the Potassium hydroxide was added and left undisturbed for a few minutes, observed for the red color.
Detection of Amino acid
Ninhydrin test: 1ml. of the extract, a few drops of ninhydrin reagent (10 mg ninhydrin in 200 ml acetone) were added to the mixture and the purple color was observed.
Detection of Terpenoids
Salkowski test: 5 ml. of plant extract was mixed with 2 mL of Chloroform, and 3 mL of concentrated sulfuric acid was carefully added to form a layer of a reddish-brown color[14].
Detection of Steroids
Chloroform+H_2SO_4 test: 2ml. Extract with 2ml. chloroform and 2ml. of concentrated H2SO4 were added as observed for the red color and yellowish-green fluorescence.
Preparation of Mobile phase for TLC plate chamber:
The extracts were assessed using TLC plates coated with 0.2 mm thick silica gel. The mobile phase used was a solvent mixture of chloroform, methanol, and ammonia in the ratio of 2:2:1 v/v. The mobile phase migrated on the silica-coated plates by capillary action and developed bands at a different distance. The fully developed coated panel was air-dried followed by heating at 45^0C for 10 minutes[15]. The spots are expressed as retention factor (Rf) calculated through the following formula:
Rf = (distance traveled by solute/distance traveled by solvent) 100;
GC-MS (Gas Chromatography-Mass Spectroscopy) analysis:
GC-MS analysis was performed on the GC-Claus 680 MS-SQ-8C PerkinElmer system, which included an AOC-20i autosampler and a gas chromatograph connected to a mass spectrophotometer (GC-MS). The sample was analyzed using the following detection parameters; Furnace initial temperature 40°C for 5 min, ramp 12°C/min to 260°C, hold 10 min, Inj B auto 250°C, volume - 0 µL, division - 50:1, carrier gas - He (99.999%), solvent delay - 2.00 min, transfer temperature 1800 °C, source temperature 200°C, scan: 50 to 500 Da[16].

Results and discussion

The preliminary phytochemical screening tests of Acetone extract of *Boerhaavia diffusa* have resulted in the presence of Carbohydrates, Amino acids, Alkaloids, Quinines,Cardiac glycosides, Phenol, Tannins, Flavonoids, Saponins, Steroids in the plant extract Table-1.
The TLC analysis reflected various distinct spots of varying intensities on the plate. (Figure: 1) While studying the plate Under 254 & 365 nm wavelength bands reflected various colors including Red, Blue, Light Pink, Sky Blue, etc. Results with Rf values are summarized in Table: 2.

Phytochemical Compounds	Tests Name	*Boerhaavia diffusa* leaf
Carbohydrates	Benedict's test	+
Alkaloids	Dragendorff's test	+
Amino acids	Ninhydrin reagent test	+
Quinines	KOH test	-
Terpenoids	Salkowski test	+
Cardiac glycosides	Keller-Killani test	+
Poly Phenol	Lead acetate test	+
Tannins	Ferric chloride test	+
Flavonoids	H_2SO_4 test	+
Saponins	Vigorously shaken of water test	+
Steroids	chloroform +H_2SO_4 test	-

Table 1: Preliminary phytochemical evaluation of acetone extracts from *Boerhaavia diffusa* leaves (+) = Present; (-) = absent

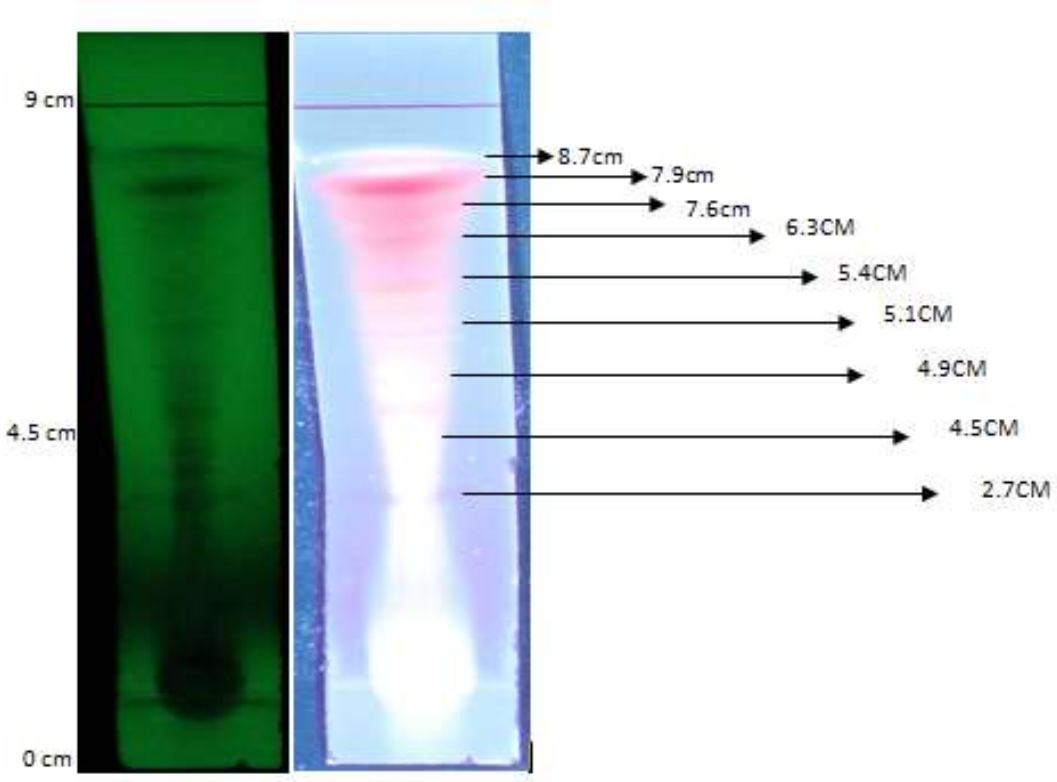

Figure 1: TLC plates visualize BDA under below 254 & 365 nm wavelength.

Sr. No.	Spot Color	Distance travelled by Solvent	Distance travelled by Solvent	Rf Value	Compounds Name
1	Blue	9 cm.	8.7 cm.	0.966	Carotene
2	Red	9 cm.	7.9 cm.	0.877	Echinenone
3	Light Pink	9 cm.	7.6 cm.	0.844	Acetophenone
4	Light Red	9 cm.	6.3 cm.	0.700	Chlorophyll a
5	Orange	9 cm.	5.4 cm.	0.600	Astaxanthin Di-esters
6	Light pink	9 cm.	5.1 cm.	0.566	Chlorophyll c
7	Radish Pink	9 cm.	4.9 cm.	0.544	p-Coumaric
8	Pink	9 cm.	4.5 cm.	0.500	Astaxanthin Monoesters
9	Sky blue	9 cm.	2.7 cm.	0.300	Astaxanthin free

Table 2: Retention factor values of *Boerhaavia diffusa* leaves to identify plant pigments.

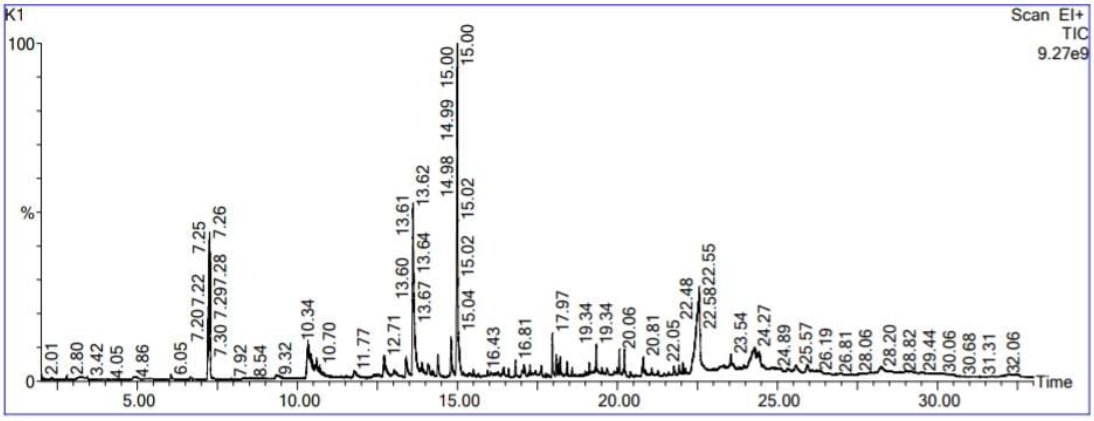

Figure 2: GC-MS spectrum of Acetone extract of *Boerhaavia diffusa*

The GC chromatogram was interpreted by MS's proprietary National Institute of Standard and Technology (NIST) database, which consists of a library of more than 7,900 compounds. The compound's details including name, molecular weight, and structure of the components present in the test materials are given in Figure: 2.

GC-MS analysis of AEBD led to the identification of multiple compounds in the plant by a mass spectrometer (MS). Comparison of the GC spectrum to the National Institute of Standards and Technology (NIST-2011) database of a library of more than 7,900 compounds led to the identification of over 200 phytochemical compounds (about 10 compounds/peak) with a total of 24 retention times (RT) peaks from the GC spectrum (Figure-1). Furthermore, on cross-verification of the reported compounds at online databases such as NCBI- Pubchem, Drug Bank, Sigma Aldrich, etc., mainly 25 compounds (fall in the 50.88% peak region of the spectrum) are included along with their known medicinal properties in Table- 3. The identified compounds include the Formamide, N-methyl-, (RT 7.245; peak area 0.926%) Propanoic acid, 2-hydroxy-2-methyl-, methyl ester, (RT 7.284; peak area 1.463%) Ethanol, pentamethyl-, (RT 7.284; peak area1.463%) Nonanal, (RT 10.337; peak area 1.990%) 2-Decenal, (E)- (RT 13.621; peak area 8.220), etc. some of

them are known for their medicinal activities like antimicrobial, antibacterial, anticancer, antitumor, Nematocidal, etc.

S. No.	RT	Peak	Compounds Name	Formula	M.W.	Biological Activity
1.	7.245	0.926	Formamide, N-methyl-	C_2H_5NO	59.07	Antioxidant activity[17]
2.	7.284	1.463	Propanoic acid, 2-hydroxy-2-methyl-, methyl ester	$C_5H_{10}O_3$	118.13	Anticancer[18]
3.	7.284	1.463	Ethanol, pentamethyl-	$C_7H_{16}O$	116.2	Antitumor[18]
4.	10.337	1.990	Nonanal	$C_9H_{18}O$	142.24	Mutagenicity study[17]
5.	10.337	1.990	2-Nonen-1-ol, (E)-	$C_9H_{18}O$	142.24	Nematicidal activity[17]
6.	10.337	1.990	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170.29	Nematicidal activity[17]
8.	10.337	1.990	2-Propylcyclohexanol	$C_9H_{18}O$	142.24	Anticancer activity[17]
9.	10.337	1.990	trans-2-Dodecen-1-ol	$C_{12}H_{24}O$	184.32	Nematicidal activity[17]
10.	10.337	1.990	Dodecanal	$C_{12}H_{24}O$	184.32	Anticancer activity[17]
11.	10.337	1.990	1-Octyn-3-ol, 4-ethyl-	$C_{10}H_{18}O$	154.25	Anticancer[17]
12.	13.621	8.220	2-Tridecenal, (E)-	$C_{13}H_{24}O$	196.33	Nematicidal activity[17]
13.	13.687	1.095	1-Pentanol	$C_5H_{12}O$	88.15	DSSTox (EPAFHM) EPA Fathead Minnow Acute Toxicity Vapors may irritate skin and eyes. Used as a solvent and to make other chemicals[18]
14.	14.392	0.906	2,4-Decadienal, (E,E)-	$C_{10}H_{16}O$	152.23	Nematicide and an apoptosis inducer[17]
15.	14.805	1.473	2-Undecenal	$C_{11}H_{20}O$	168.28	Antimicrobial activity[18]
16.	15.007	12.482	7-Tetradecenal, (Z)	$C_{14}H_{26}O$	210.36	Fatty Aldehyde[18]
17..	15.007	12.482	cis-11-Hexadecenal 53939-	$C_{16}H_{30}O$	238.41	identify small molecule agonists of the estrogen receptor alpha (ER-alpha) signaling pathway using the BG1 cell line[18]
18	19.339	0.853	9-Octadecenal, (Z)-	$C_{18}H_{34}O$	266.5	Enables fatty acid amide hydrolase activity, flavoring Agent[18]
19.	22.553	11.028	cis-Vaccenic acid	$C_{18}H_{34}O_2$	282.5	Telomerase activity[17]
20.	23.550	0.989	Oxacycloheptadec-8-en-2-one, (8Z)	$C_{16}H_{28}O_2$	252.39	Personal care, flavoring, perfuming, and fragrance[18]
21.	24.300	3.757	Oleic Acid	$C_{18}H_{34}O_2$	282.5	Antifoaming agents, flavoring agents, the preparation of cleaning agents, and lotions[18]
22.	24.300	3.757	Palmitoleic acid	$C_{16}H_{30}O_2$	254.41	Human blood serum metabolite[18]
23.	24.409	2.006	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	Saturated fatty acids come from many animal and vegetable fats and oils[18]
24.	24.409	2.006	Eicosanoic acid	$C_{20}H_{40}O_2$	312.5	Transcriptional activity[18]

25.	28.259	2.904	cis-13-Eicosenoic acid	$C_{20}H_{38}O_2$	310.5	Mouse metabolite, a rat metabolite, and a plant metabolite[18]
26.	28.259	2.904	trans-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282.5	Human metabolite[18]

Table 3: Detail of compounds identification from GC-MS analysis of an acetone extract of *Boerhaavia diffusa.*

The identification of Flavonoids, Terpenoids, steroids, Tannin, Carbohydrates, Glycosides and Phenolic compounds in the acetone extracts of *Boerhaavia diffusa*has established the medicinal importance of the plant as these agents determine antibacterial, anti-cancer, anti-inflammatory, and antioxidant properties of the plant[19]. This indicates that the plant contains a range of chemical components that may be responsible for many pharmacological effects and have protective or disease-preventing properties. Alkaloids are beneficial chemicals for plants with anti-predatory and anti-parasitic effects[20]. The polyphenols possess anti-parasitic activity and monoterpenes have been reported to produce anti-plasmodic, anti-neoplastic and anti-viral activities[21]. Mahesh et al in their study on the whole plant in 2012 reported the anti-bacterial, hepatoprotective, hypoglycemic, anti-estrogenic, anti-inflammatory, anti-stress, and anti-metastatic activities of the plant[22]. The phytochemical profiling of the plantin our study and by other researchers also provides insight into its value as a medicinal as well as a nutritious plant. As it has not shown any toxic compounds hence, it may be safe for consumption both as medicine and as a natural source of antioxidant and antioxidant-promoting activities.

The detection of about 25 major compounds like Formamide, N-methyl- (RT 7.245; peak area 0.926%) Propanoic acid, 2-hydroxy-2-methyl-, methyl ester (RT 7.284; peak area 1.463%) Ethanol, pentamethyl- (RT 7.284; peak area1.463%) Nonanal (RT 10.337; peak area 1.990%) 2-Decenal,(E)- (RT 13.621; peak area 8.220)in our study through GC-MS analysis has revealed the added medicinal value such as antimicrobial, antibacterial, anticancer, antitumor, Nematicidal, etc. activity of the plant. Moreover, the identification of compounds likeOctadecanoic acid(RT24.409; peak area 2.006%),3-Nonen-1-ol, (Z)-(RT 10.337; peak area 1.990%),Oleic Acid (RT 24.300; peak area 3.757%),Oxacycloheptadec-8-en-2-one, (8Z) (RT 23.550; peak area 0.989%)The added medicinal value such as Saturated fatty acid, food & flavoring agent, Anti-foaming agent, personal care, perfuming and fragrance. It has established the industrial value of the plant. The presence of various bioactive compounds in *Boerhaavia diffusa* justifies the use of the whole plant for various ailments by traditional practitioners. Further, a detailed study of various compounds present in *Boerhaavia diffusa* and their pharmacological evaluation may help in developing drugs for Cancer and other microbial diseases with multiple effects.

Conclusions:

The identification of various phytochemical compounds in the acetone extract of *Boerhaavia diffusa* has revealed additional medicinal properties of the plant. In addition to its known effects, the plant is found to possess compounds that hold Antimicrobial, Antibacterial, anti-cancer, Transcriptional activity, Nematocidal, and Antioxidant properties. Since the plant is also a good source of human metabolites, food additives, and fragrances, therefore it can also be a good agent for the food & cosmetics industry. Further isolation, purification, and characterization of unknown compounds may reveal more pharmacological values of the plant.

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18. PubChem Database. Explore chemistry. Natl Cent Biotechnol Information [Internet]. (2022);1–2. Available from: Propanoic acid, 2-hydroxy-2-methyl-, methyl ester, Ethanol, pentamethyl-, 2-Tridecenal, (E)-, 1-Pentanol, 2-Undecenal, 7-Tetradecenal, (Z)-, cis-11-Hexadecenal 53939-, 9-Octadecenal, (Z)-, cis-Vaccenic acid, Oxacycloheptadec-8-en-2-one, (8Z), Oleic Acid.
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