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Phyto-Chemical Study of Bark Extract of *Nyctanthus Arbortristits* LINN. Belonging to Family Oleaceae

Research Article

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Abstract

Nyctanthus arbortristis, a commonly known as Parijatak, has traditionally been used in treatment of rheumatic joint pain, malaria and used as an expectorant. The stem bark is recommended for curing periodic fever. This study aims to the characterization of the bioactive constituents from extract of *Nyctanthus arbortristis* in Petroleum ether, methanol using UV-VIS, FTIR and NMR. Compounds isolated from *Nyctanthus arbortristis* were identified are (MNA-I – fraction of ethyl acetate: methanol -1:1) as a 3-Ethoxypropionaldehyde diethyl acetal, 1-Eicosene, 1-Hexadecanol, 2-methyl- and (MNA-II - fraction of ethyl acetate: methanol -3:7) as a 3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone.

Keywords: *Nyctanthus arbortristis,* Bark, 1-Eicosene, 1-Hexadecanol

Abbrevations

PENA: Petroleum ether extract of Nyctanthus arbortristis; MNA: Methanolic extract of Nyctanthus arbortristis; MNA-I: Methanolic extract of *Nyctanthus arbortristis* - Ethyl acetate: Methanol (50:50); MNA-II: Methanolic extract of *Nyctanthus arbortristis* - Ethyl acetate: Methanol (30:70)

Introduction

India is one of the world's 12 biodiversity centers with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different Agri-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). However, only 7000-7500 species are used for their medicinal values by traditional communities. In India, drugs of herbal origin have been used in traditional systems of medicines such as Unani and Ayurveda since ancient times. In the foregoing works of wisdom by renowned Ayurvedic experts Charak have been proved repeatedly by human experience all over the world. Man since time immortal has been using herbs or plant products as medicine for developing immunity or resistance against cold, joint pain, fever etc. In 1994 Gupta & et.al reported as a vast majority of our population, particularly those living in villages depend largely on traditional remedies. In 2004, Kokate & et.al reported, the nature has provided a complete storehouse of remedies to cure all ailments of mankind. Since the down of civilization, in addition to food crops, man cultivated herbs for his medicinal needs. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature [1-4].

Phytochemistry is the study of phytochemicals produced in plants, describing the isolation, purification, identification, and

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structure of the large number of secondary metabolic compounds found in plants. Effect of extracted plant phytochemicals depends on:

- The nature of the plant material
- Its origin
- Degree of processing
- Moisture content
- Particle size

Carbon dioxide gas deals with the photosynthesis process in plants in the presence of light energy. Photosynthesis and pentose pathway together pools the phosphate group present in the sugar molecules of plants which leads to glycolysis process and which is accounted for producing many of phytochemicals of plants, such as, shikimic acid, proteins, aliphatic and aromatic acids, mevalonic acids, fatty acids, flavanoids, terpinoids, steroids etc. There are lots of medicinal plants which contain a number of phytochemicals and those phytochemicals are used medicine purpose to treat various kinds of diseases. In the following table a list is shown of phytochemicals having medicinal values [5,6].

Importance of Phytoconstituents: Therapeutic potential of plant and animal origin are being used form the ancient times by the simple process without isolation of pure compounds that is in the form of crude drugs or galanicals prepared from them. The pharmacological action of crude drug is determined by the nature of its constituents. Thus, the plant species may be considered as a biosynthetic laboratory not only for the chemical compounds e.g., carbohydrates, proteins and fats that are utilized as food by humans and animals, but also for the multitude of the compounds including alkaloids, triterpenoids, flavonoids, glycosides etc. which exert definite physiological effects.

Generally chromatographic techniques are the most useful tool for such purpose. Thin layer chromatography (TLC), preparative TLC, column chromatography, HPLC, gas chromatography and HPTLC are the various techniques for the separation and isolation of the phytoconstituents. Spectral analysis of such isolated constituents by using following techniques as UV spectroscopy, IR spectroscopy, GC - MS can be applied, NMR spectroscopy.

Table 1: Structural features and activities of various phytochemicals from plants.

Phytochemicals	Structural features	Example(s)	Activities
Phenols and Polyphenols	C3 side chain, -OH groups, phenol ring	Catechol, Epicatechin, Cinnamic acid	Antimicrobial, Anthelmintic, Antidiarrhoeal
Quinones	Aromatic rings, two ketone substitutions	Hypericin	Antimicrobial
Flavones Flavonoids Flavonols	Phenolic structure, one carbonyl group Hydroxylated phenols, Flavones + 3-hydroxyl group	Abyssinone Chrysin, Quercetin, Rutin Totarol	Antimicrobial, Antidiarrhoeal
Tannins	Polymeric phenols (Mol. Wt. 500-3000	Ellagitannin	Antimicrobial, Anthelmintic
Coumarins	Phenols made of fused benzene and α-pyrone rings	Warfarin	Antimicrobial
Terpenoids and essential oils	Acetate units + fatty acids, branching	Capsaicin	Antimicrobial, Antidiarrhoeal
Alkaloids	Heterocyclic nitrogen compounds	Berberine, Piperine, Palmatine, Tetrahydropalmatine	Antimicrobial, Anthelmintic, Antidiarrhoeal
Lectins and Polypeptides	Proteins	Mannose-specific agglutinin,Fabatin	Antidiarrhoeal

Standardization of Phytoconstituents: Organoleptic characteristics, specific chemical tests, macroscopic analysis, pharmacognostic parameter, microscopic analysis & comparison using crude drug reference standard are conducted. After the proper identification, the purity of herb should be assessed by way of detection of foreign organic matter (i.e. insects, rodent debris etc.), detection of foreign inorganic matter (i.e. total ash, acid insoluble ash etc.) [7].

Materils & Methods

A) Plant Material

a) Collection and Drying: The Bark of *Nyctanthes arbortristis* belonging to family Oleaceae was taken for present study based on the literature survey. The crude drug was collected from Khadakewake village, Tahasil Rahata, Ahamednagar district (M.S, INDIA).

b) Plant Authentication: The plant was authenticated by P. G. Diwakar, Joint Director, Botanical survey Of India, Pune by comparing morphological features. The herbarium of the plant specimen was deposited at Botanical Survey of India, Pune.

B) Preparation of *N. arbortristis* extracts:

A dried material was extracted with different solvents, starting from solvent of low polarity. Initially crude drug was extracted with petroleum ether (60-80^{0}c), furthermore was extracted with methanol.

Briefly, 500 gm powdered bark of *Nyctanthes arbortristis* was packed in thimble containing cotton cloth and extracted with petroleum ether (60-80^{0}c) in soxhlet apparatus. After complete extraction, filtrate was filtered off and solvent was recovered using distillator. The extract was concentrated to dry residue and kept in desiccator over adsorbent like sodium sulphate. Further, marc was extracted using methanol.

a) Preparation of Petroleum ether dried extract: The shade dried, coarse powder of the stem bark of *Nyctanthes arbortristis Linn.* (500gm) was packed well in a soxhlet apparatus and extracted with petroleum ether (60-80°C) until the extraction was completed which was confirmed by the color of the siphoned liquid. The extract was filtered while hot and the resulting extract was distilled in vacuum in order to remove the solvent completely and subsequently dried in a desiccator. The extract was weighed and calculated the percentage yield in terms of air-dried material.

b) Preparation of Methanol dried extract: The marc was dried in hot air oven below 50°C and packed well in Soxhlet apparatus and extracted with methanol until the completion of the extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely and dried in a desiccator. Weighed the extract and calculated its percentage in terms of air-dried powdered crude material.

C) Preliminary Phytochemical Investigation:

The extract obtained after extraction was characterized by

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preliminary phytochemical test for rough ideas of main constituents present in extracts. Alkaloid, Cardiac glycosides, Tannin, Terpenoid, Phlobatannins, Fixed oils and fats and Flavonoid in the petroleum and methanol extracts of the bark of N. arbortristis were identified.

D) Separation & isolation of phytoconstituents by Chromatography

Thin layer chromatography (TLC) and Column chromatography technique is used for separation, isolation and identification of constituents. First of all, TLC of different extracts was performed with suitable solvent systems, different solvents were used to separate out different chemical constituents. The TLC for PENA and MNA was performed. Then, for isolation of the phytoconstituents, the methanol extract was fractionated by the column chromatography.

Thin layer chromatography: thin layer chromatography (TLC) technique is used as a base for separation, isolation and identification of constituents. First of all, TLC of different extracts was performed in different solvent systems, for selection of optimum mobile phase. Different solvents were used to separate out different chemical constituents. The TLC for PENA and MNA was performed [8].

i. Thin layer chromatography of petroleum ether extract of bark of *N. arbortristis*

- **Stationary phase:** Precoated silica plates (E. Merk)
- **Mobile phase:** Benzene: Ethyl acetate (9:1)
- **Visualization:** 1. Spray Vanilline – Sulphuric acid reagent and heat at 105^{0}C

ii. Thin layer chromatography of methanol extract of bark of *N.arbortristis*

- **Stationary phase:** Precoated silica plates (E. Merk)
- **Mobile phase:** Ethyl acetate: Formic acid: Glacial acetic acid: Water (100:11:11:26)
- **Visualization:** Spray Anisaldehyde - Sulphuric acid reagent and heat at 105^{0}C

Column Chromatography: To isolate the phytoconstituents responsible for the activity, the methanol extract was fractionated by the column chromatography.

- **Height of column:** 30 cm
- **Diameter of column:** 3 cm
- **Stationary phase:** Silica for column chromatography
- **Mobile phase:** gradient elution method

1. Ethyl acetate
2. Methanol

Detection of spot: By spraying vanillin sulphuric acid reagent and heating at 110^{o}Cfor 10 min.

No. of fractions collected: 20

Volume of each fraction: 25ml

E) Phyto-chemical screening by Spectroscopic methods

UV-visible spectrophotometric analysis was conducted on the *N. arbortristis* extract using a UV-visible spectrophotometer with a slit width of 2nm, using a 10-mm cell at room temperature. The extract was examined under visible and UV light in the wavelength ranging from 300-600nm for proximate analysis. For UV-VIS spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and filtered through Whatman No. 1 filter paper. The sample is diluted to 1:10 with the same solvent. Fourier transform infrared (FTIR) was used to identify the characteristic functional groups in the extract. It provides the information about the structure of a molecule could frequently be obtained from its absorption spectrum. A small quantity of the Mentha spicata extract was mixed in dry potassium bromide (KBr). The mixture was thoroughly mixed in a mortar and pressed at a pressure of 6 bars within 2 min to form a KBr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Bruker, Germany Vertex 70 infrared spectrometer. The sample was scanned from 4000 to 400 cm^{-1}. The peak values of the UV-VIS and FTIR were recorded. NMR spectra were obtained from a Bruker spectrometer using tetramethyl silane (TMS) as an internal standard in DMSO. Chemical shifts were in ppm concerning TMS. Coupling constants were in Hz [9-15].

Results

Profile of *Nyctanthes Arborstristis*

Figure 1.1: Image of *Nyctanthes Arborstristis.*

Figure 1.2: Image of *Nyctanthes Arborstristis.*

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Common Name: Parijatak (sad tree)

Botanical Name: *Nyctanthes arbortristis*

Vernacular Names: English - Night-flowering Jasmine, Coral Jasmine

Hindi - Harashringara, Harsingar

Bangali - Sephalika

Nepali - Parijata, Paghala

Sanskrit – Parijatha

Malayalam – Parijatakam

Gujarathi – Jayaparvati

Kannada - Parijatha

Telugu – Pagadamalle

Oriya – Gangasiuli.

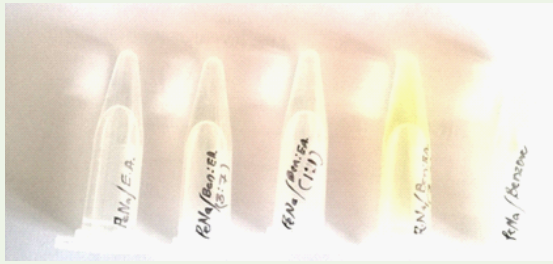
Plant authentication is done with Botanical survey of India, regional office Pune.

A) Preparation of *N. arbortristis* extracts: The extraction values of *N. arbortristis* is as follow

a) Preparation of Petroleum ether dried extract:

Table 1: Petroleum ether extract of *N. arbortristis*.

S.No.	Extract	Color	Nature	Extractive value(%w/w)
1	Petroleum Ether extract of bark of *Nyctanthes arbortristis*	Dark brown	Semi-solid	1.4



b) Preparation of Methanol dried extract:

Table 2: Methanol extract of *N. arbortristis*.

S.No.	Extract	Color	Nature	Extractive value (%w/w)
1	Methanol extract of bark of *Nyctanthes arbortristis*.	Green	Semi-solid	6.5

B) Preliminary Phytochemical Investigation:

C) Isolation & separation of phytoconstituents by Chromatography:

a) Thin Layer Chromatography:

- Thin layer chromatography of petroleum ether extract of bark of *N. arbortristis*
- Thin layer chromatography of methanol extract of bark of *N. arbortristis*

b) Column Chromatography:

Table 3: It shows phytochemical screening of petroleum ether and methanol extracts of *N. arbortristis* (PENA & MNA).

S. No.	Chemical constituent	Chemical test	PENA	MNA
1	Alkaloids	Dragendorff's test Mayers test	- -	+ +
2	Steroids	Salkowaski test Liebermann-burchard test	+ +	- -
3	Triterpene	Vanillin-sulphuric acid test	+	-
4	Tannin	Ferric chloride test Dilute nitric acid test	- -	+ +
5	Glycoside	Keller-killani test	-	+
6	Carbohydrate	Molish test Fehling's test	- -	- -
7	Flavonoid	Shinoda test Lead acetate test	- -	+ +
8	Saponins	Foam formation test	-	-
9	Protein	Biuret test Millon's test	- -	- -
10	Amino acid	Ninhydrin test	-	-

Figure 2: TLC of petroleum ether extract of bark of N. arbortristis visualized by spraying vanilline – sulphuric acid reagent.

Figure 3: TLC of methanol extract of bark of *N. arbortristis* visualized byspraying anisaldehyde-sulphuric acid reagent.

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Table 4: Rf values of phytoconstituents from petroleum ether extract of bark of *N. arbortristis* in TLC

S. No.	Bark extract	
	Rf	Remark
1	0.3	Violet colour
2	0.4	Violet colour
3	0.44	Violet colour
4	0.79	Blue colour

Table 5: Rf values of phytoconstituents from methanol extract of bark of *N.arbortristis* in TLC.

S. No.	Bark extract	
	Rf	Remark
1	0.06	Blue colour
2	0.24	Blue colour
3	0.29	Blue colour
4	0.35	Blue colour
5	0.51	Blue colour
6	0.69	Blue colour

Table 6: Fractionation of Methanol extract of bark by column chromatography.

Solvent used in Column	No. of Fractions	Colour of Fraction	Inference	Code
Ethyl Acetate	04	Faint green	Not processed	
Ethyl Acetate: Methanol (70:30)	04	Green	Not processed	
Ethyl Acetate: Methanol (50:50)	04	Brownish green	Processed for purification	MNA-I
Ethyl Acetate: Methanol (30:70)	04	Green	Processed for purification	MNA-II
Methanol	04	Greenish yellow	Not processed	

IR spectra: (MNA-I fraction)

Table 7: IR spectra of MNA-I

S. No.	Wave number (cm^{-1})	Functional group
1	1056	C-O esters
2	1378	C-H methyl
3	1722	C=O esters
4	3635	O-H alcohols, phenols

IR spectra: (MNA-I fraction)

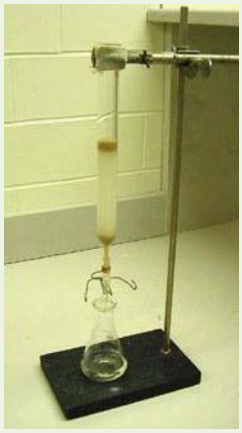

Figure 4: Column chromatography.

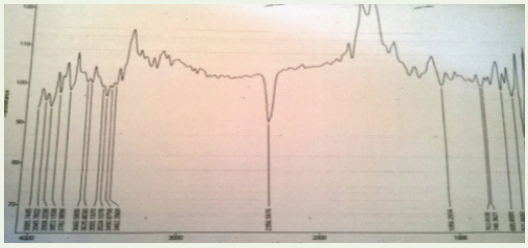

Figure 5: IR of MNA-I.

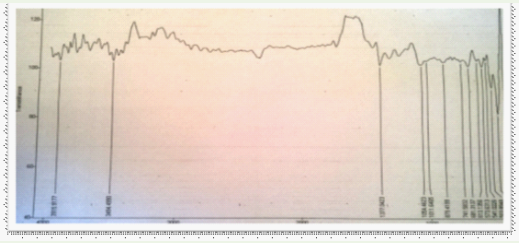

Figure 6: IR of MNA-II.

Table 8: IR spectra of MNA-II.

S.No.	Wave number (cm^{-1})	Functional group
1	1054	C-O esters
2	1377	C-H methyl
3	1722	C=O esters
4	3494	O-H alcohols, phenols

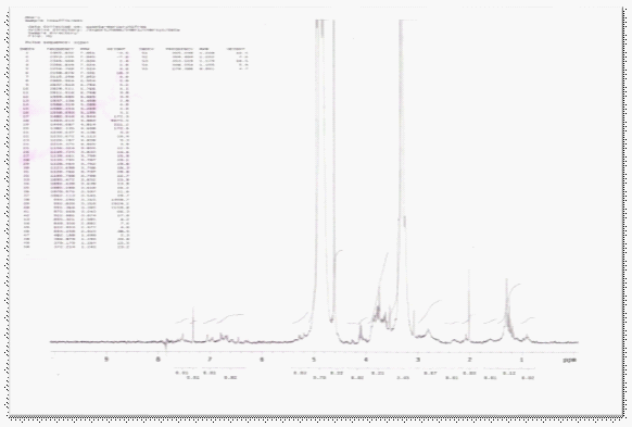

Figure 7: NMR of MNA-I.

Table 9: NMR of MNA-I.

S. No.	Frequency	PPM	Height
1	1482.944	4.944	172.3
2	1464.615	4.883	9675.5
3	1444.087	4.814	211.2
4	994.296	3.315	1998.7
5	992.830	3.310	2624.1
6	991.364	3.305	2159.0
7	972.668	3.243	66.3
8	604.258	2.015	38.5

NMR spectra: (MNA-II fraction)

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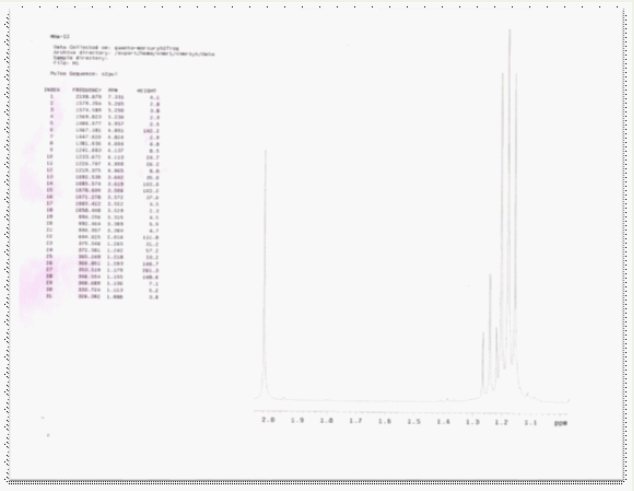
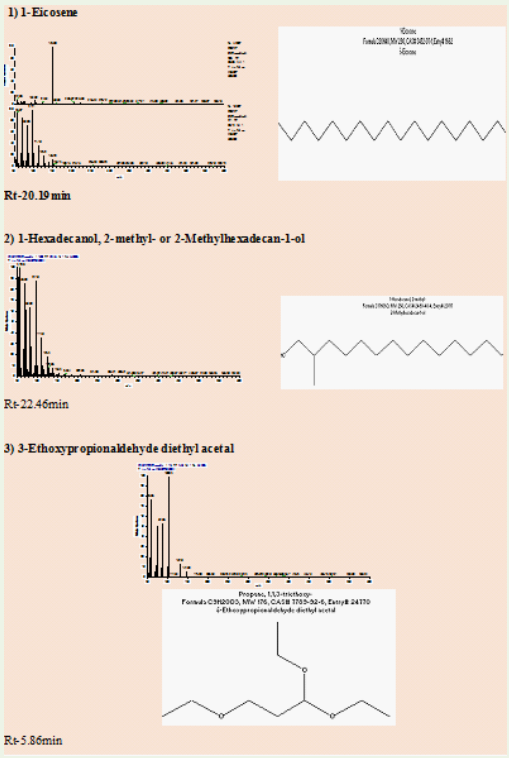


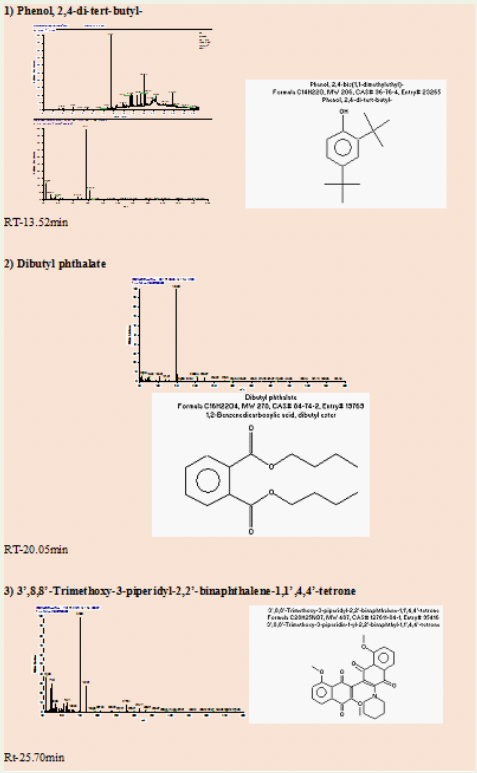
Figure 8: NMR of MNA-II.

Table 10: NMR of MNA-II.

S. No.	Frequency	PPM	Height
1	1467.181	4.891	142.2
2	1085.574	3.619	102.0
3	1078.609	3.596	102.2
4	604.625	2.016	111.8
5	360.851	1.203	146.7
6	353.519	1.179	261.3
7	346.554	1.155	148.6



From MNA-I fraction



From MNA-II fraction.

Table 11: Compound identified in following methanol extract fractions.

S. No.	Name of compound (From MNA-I fraction)	Retention Time	Mol. Formula	Mol. Weight
1	1-Eicosene	20.19	C20H40	280
2	1-Hexadecanol, 2-methyl- or 2-Methylhexadecan-1-ol	22.46	C17H36O	256
3	3-Ethoxypropionaldehyde diethyl acetal	5.86	C9H20O3	176
S. No.	**Name of compound (From MNA-II fraction)**	**Retention Time**	**Mol. Formula**	**Mol. Weight**
1	Phenol, 2,4-di-tert-butyl-	13.52	C14H22O	206
2	Dibutyl phthalate	20.05	C16H22O4	278
3	3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	25.70	C28H25NO7	487

Discussion

In the present study, we found that phytoconstituents in different fractions separated through column chromatography. Ethyl acetate: methanol (1:1) & ethyl acetate: methanol (3:7) fractions were processed [16].

Conclusion

In present study, compounds isolated from this species were identified by analytical tools like, I.R, GC-MS and NMR are (MNA-I – fraction of ethyl acetate: methanol -1:1) as a 3-Ethoxypropionaldehyde diethyl acetal, 1-Eicosene, 1-Hexadecanol, 2-methyl- and (MNA-II - fraction of ethyl acetate: methanol -3:7) as a 3',8,8'-Trimethoxy-3-piperidin-1-yl-2,2'-binaphthyl-1,1',4,4'-tetrone.

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