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Isolation and Identification of Terpenoids and Sterols of *Nepeta cataria* L.

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Abstract: Chemical analysis of the air dried flowering aerial parts of *N. cataria* showed moisture (6.2%); ash (7.9%); crude fiber (15.57%); crude protein (9.13%); crude lipid (4.88%) and carbohydrate (62.5%).Fixed oil extracted from the air dried flowering aerial parts of *N. cataria* contained lauric (3.7%); myristic (7.2%); palmitic (20.3%); stearic (18.6%); arachidic (4.1%); palmitoleic (9.6%); oleic (14.2%); linoleic (9.3%) and linolenic (5.8%) in sap part and unsap contained dodecane (3.95%);α-tocopherol (5.3%); pentacosane (0.84%); hexacosane (10.16%); nonacosane (6.83%); henetricontane (26%); dotriacontane (2.98%) and. β-sitosterol (18.6%); stigmasterol (8.9%) and campsterol (6.52%). In identification of terpenoids and sterols of petroleum ether extract (40-60) of *Nepeta cataria* L**,** four major compounds 1, 2, 3 and 4 were isolated by column chromatography; according to their order of elution. Their spectral characters proved them to be α- amyrine, ixoroside aglycone, β sitosterol and ursolic acid.
Key words: *Nepeta cataria*, sterols and triterpenes , fatty acids.

Introduction

The perennial herb, *Nepeta cataria* L. is a member of the mint family, Lamiaceae; order, Lamiales; var. *citriodora* (Becker) Balb)[1]. The genus *Nepeta* (Lamiaceae) comprises about 400 species, most of which grow wild in Central and Southern Europe, North Africa and Central and Southern Asia[2,3,4] . Commonly known as "Catnip or Catmint" because of its irresistible action on cats. Due to lemony mint flavor it finds the ways in the herbal teas as well as in cooking, and has a considerable folkloric reputation. In traditional use, catnip is believed to have sedative, carminative, antispasmodic properties and to treat colds, flu, and fevers[5,6]. Medicinally, the plant is used in gastrointestinal and respiratory hyperactive disorders such as colic, diarrhea, cough, asthma and bronchosis[7,8], and treating cardiovascular complaints, such as angina pectoris, cardiac thrombosis, tachycardia, and heart weakness[9]. Several classes of secondary metabolites have been isolated such as flavonoids, phenolic compounds, essential oil-containing monoterpenes, sesqterpenes, and sterols[10,11,12,13,14]. Rosmarinic acid, an ester of caffeic is one of the main active constituents of *N. cataria*. In addition, the plant

also contained nepetalactones and alkaloids, such as, actinidine and iridomyrmecine[15]. *Nepeta* is also reported to possess biological activities; include antibacterial[15], antifungal[16], analgesic[17]and behavioral[18] and reduction of serum lipids and anti-inflammatory effects[19,20].

Materials and Methods

Plant materials

Seeds of catnip (*Nepeta cataria* L.) were obtained from company of Jelitto Staudensamen, Schwarmstedt, Germany. The aerial parts of (*Nepeta cataria* L.) were collected from plants cultivated in the Experimental Farm of the National Research Centre, Nobaria, El-Bihara Governorate (150 Km Northern South of Cairo), Egypt, air dried and fine-powdered.

Solvents

All solvents and chemicals were of analytical grade.

Preliminary phytochemical screening of *Nepeta cataria*

The powdered air-dried aerial parts of *Nepeta cataria* were phytochemically screened[21].

Proximate analysis

Analysis of crude protein, crude fiber, ash, moisture and crude lipid as a proximate analysis of the aerial parts of *Nepeta cataria* were determined[22], and also carbohydrates.

Preparation of different plant extracts

The powdered air-dried aerial parts of *Nepeta cataria* (720 g) were subjected separately to exhaustive continuous successive extraction using Soxhlet apparatus. Using petroleum ether (40-60 ^{o}C), chloroform, ethyl acetate and ethanol (80%). Also the powdered whole air-dried aerial parts of *Nepeta cataria* (400g) were extracted with 70% ethanol. The extracts petroleum ether chloroform, ethyl acetate and ethanol (80%), beside 70 % ethanol extract of the whole air-dried aerial parts of the plant in succession, afforded 35, 28, 4.5 and 29 g in addition, 26g of dried total ethanol extracts, respectively.

Separation of fatty acids and unsaponifiable matters of *Nepeta cataria*

Saponification of petroleum ether extract was carried out [22]. The unsaponifiable matters were extracted three times with light petroleum ether. The aqueous layer was acidified by HCl (20% w/v), and the liberated fatty acids were extracted with petroleum ether. The separated extracts of unsaponifiable matters and fatty acids were washed several times with distilled water and dried over anhydrous sodium sulfate**.**

Methylation of fatty acids

Fatty acids of standards and samples were converted to methyl esters using etherial solution of diazomethane[23].

GC/MS spectroscopy of the fatty acids methyl ester

GC/MS analysis was performed a capillary GC/MS: a Agillant HP 5973 mass-spectrometer (Electron impact ionization, 70 electron voltage) coupled to 6890 gas chromatography fitted with TR-5MS (5% Phenyl Polysil Phenylene Siloxane) capillary column (Thermo scientific, 0.25 mm x 30m, 0.25 μm film thickness). The injector temperature : 200 ºC, decretory temperature 220 ºC, and temperature programming was maintained at 140 ºC for 5 min, elevated to 200 ºC at a rate of 5 ºC/min and hold time 3 min. Helium was used as the carrier gas at a flow rate of 1m L/min and samples were introduced using a column injection mode.

GC/MS spectroscopy of the unsaponifiable matters

GC/MS analysis was performed in capillary GC/MS : a Agillant HP 5973 mass-spectrometer (Electron impact ionization, 70 electron voltage) coupled to 6890 gas chromatography fitted with TR-5MS (5% Phenyl Polysil Phenylene Siloxane) capillary column (Thermo scientific, 0.25 mm x 30m, 0.25 µm film thickness). The injector temperature: 270 ºC, decretory temperature 280 ºC, and temperature programming was maintained at 70 ºC for 5 min, elevated to 280 ºC at a rate of 5 ºC/min and hold time 2 min. Helium was used as the carrier gas at a flow rate of 1m L/min and samples were introduced using a column injection mode.

Chromatography of petroleum ether and chloroform extracts of *Nepeta cataria*

The petroleum ether and chloroform extracts were submitted to TLC (silica gel) using solvent systems, benzene: ethyl acetate (60 : 20) and, benzene: ethyl acetate (80:20), and benzene: ethyl acetate (90 : 10)[12]; then sprayed with sulphuric acid (10%) and heated at 105ºC for few seconds. Seven spots (Rf's; 0.084, 0.18, 0.44, 0.65, 0.79, 0.86 and 0.93) for petroleum ether extract and for chloroform extract were given five spots (Rf's; 0.11, 0.42, 0.66, 0.81 and 0.88).

Column chromatographic separation of petroleum ether extract

Petroleum ether extract was the most potent extract[24,25], so it was fractionated by column chromatography. 17g of dry petroleum ether extract were fractionated by column chromatography (120 cm length and 5.5 cm diameter) packed with 500 g Kieselgel (60), MERCK (0.063-0.2 mm). Elution was carried out by petroleum ether 100%, followed by petroleum ether with increasing ratios of chloroform (90:10, 80: 20,70 : 30, 60:40, 50 :50 then chloroform 100%), followed by chloroform : methanol mixture (90 :10, 80 :20, 70 : 30, 60:40, 50 : 50 followed by 100% methanol). Fractions of 50 ml, each were collected, concentrated and monitored by TLC similar fractions were mixed and the major compounds were purified on silica gel aluminum sheets. Using benzene: ethyl acetate (90:10) and kept for identification.

Results and Discussion

Preliminary phytochemical screening of the dried aerial parts of *Nepeta cataria* L. in the flowering stage

Data presented in Table (1) Showed volatile oil, tannins, sterols and / or triterpenes and flavonoids. While alkaloids and nitrogenous bases were found traces, coumarins, saponins and Anthraquinones were absent in *Nepeta cataria* L. These results are in agreement with those on *Nepeta septemcrenata* Erenb[26].

Data presented in Table (2) demonstrate the preliminary phytochemical screening of different successive extractives and 70 % ethanolic extract of the dried aerial parts of *Nepeta cataria* in the flowering stage. Sterols and /or triterpenoid compounds were detected in the petroleum ether, chloroform and 70% ethanol extracts. Ethyl acetate, ethanol as successive and 70% ethanolic extracts contain flavonoids. These results are in compatible with cited literature[10,11,12,13,14]

Table1. Results of preliminary phytochemical screening of *Nepeta cataria* L.

Test	Result
1-Volatile oil	+
2- glycosides and / or carbohydrates	+
3-Coumarins	-
4-Tannins (FeCl3 test)	+
5-Flavonoids (Shinoda's test)	+
6- Saponins (Frothing test)	-
7-Sterols and or triterpenes (Libarman-Burchard's test)	+
8-Alkaloids and/or nitrogenous bases	-
9-Anthraquinones (free and combined)	-

(+) = present; (-) = absent

Table 2. Percentage and chemical properties of successive extractives and 70% ethanolic extract of *Nepeta cataria* L

Extracts	Petroleum	Chlorofor	Ethyl	Ethanol	Ethanol
Percentage(%)	4.9	3.9	0.63	4.03	6.5
II Phytochemical screening 1-Sterol and/or triterpenes	+	+	-	-	+
2-Tannins	__	__	+	+	+
3-Coumarins	__	__	__	-	-
4-Flavonoids	__	__	+	+	+

(-) Absent , (+)Present. , *Average of three determinations

Table 3. Proximate analysis of air dried flowering aerial parts of *Nepeta cataria* and *Nepeta suaves* L

Constant	*Nepeta cataria* (%)	*Nepeta suaves* (%)
carbohydrate	62.5	20.28
Crude protein	9.13	4.7
Moisture	6.2	8.45
Total ash	7.90	7.92
Crude fiber	15.57	40.15
Crude lipid	4.88	12.6

*Average of three determinations

The results were more or less similar or show some difference from another species *Nepeta suaves*[27].

The fatty acids composition of the air dried flowering aerial parts of *Nepeta cataria* L.

Data in Table (4) show the fatty acids composition of the flowering air dried aerial parts of *Nepeta cataria* by using GC/MS mentioned in the experimental part. It is clear that the unsaturated fatty acids include oleic acid (18:1) (14.2%) followed by palmitoleic (16:1) (9.6%) and linoleic(18:2) (9.3%) and linolenic (18:3) (5.8%). While, the saturated fatty acids are palmitic acid (16:0) (20.3%), stearic acid (18:0) (18.6%), myristic acid (7.2%), arachidic acid (20:0) (4.1%), docosanoic acid (22:0) (3.8%), lauric acid (12:0) (3.7%), tetracosanoic acid (24:0) (2.3%) and hexacosanoic acid (26:0) (1.1%). No results of fatty acid composition of air dried aerial parts of *Nepeta cataria L.* are found in review of literature. [26] analyzed the fatty acid composition of air dried plant of *Nepeta septemcrenata* Erenb contained stearic acid (18:0) most abundant as the saturated fatty acid (20.1%), palmitic acid (16:0) (18.7%), myristic acid (14:0) (9.26%), Lauric acid (12:0) (5.84%), capric acid (10:0) (3.92%), caprilic acid (8:0) (2.31%) and arachidic acid (20:0) (0.74%). While the unsaturated fatty acids were linoleic (18:2) (14.37%), oleic acid (18:1) (11.26%), palmitoleic acid (16:1) (10.52%), arachidonic acid (20:4) (1.65%) and myristoleic acid (14:1) (1.12%).

Table 5. Fatty acid composition of the air dried flowering aerial parts of *Nepeta cataria* L.

Fatty acid	Degree of unsaturation	Relative %
Lauric acid	C 12: 0	3.7
Myristic acid	C14:0	7.2
Palmitic acid	C16:0	20.3
Stearic acid	C18:0	18.6
Arachidic acid	C20:0	4.1
Docosanoic acid	C22:0	3.8
Tetracosanoic acid	C24:0	2.3
Hexacosanoic acid	C26:0	1.1
Palmitoleic	C16:1	9.6
Oleic acid	C18:1	14.2
Linoleic acid	C18:2	9.3
Linolenic acid	C18:3	5.8
Total Saturated fatty acids		61.1
Total mono unsaturated fatty acids		23.8
Total poly unsaturated fatty acids		15.1

The unsaponifiable matter of *Nepeta cataria* L.

The unsaponifiable matter of the flowering air dried aerial parts of *Nepeta cataria* was analyzed using GC/MS as mentioned before. Data in Table (6) illustrate that n-henetricontane (26%) is the major hydrocarbons followed by n-hexacosane (10.16%), n-nonacosane (6.83 %), α-tocopherol (5.3%), n- dodecane (3.95 %), n-dotricotane (2.98%), n-heptacosane (2.7%), n-triatriacontane (2.57%), n-triacontane (1.91%) and squalene (0.68%) in descending order, in addition to β- sitosterol (18.6%), stigmasterol (8.9%), campesterol (6.52%) and α- amyrine (0.14%). No results of unsaponifiable matter of air dried flowering aerial parts of *Nepeta cataria L.* are found in review of literature. However, unsaponifiable matter of *Nepeta septemcrenata* Erenb contained 12 hydrocarbons; dodecane (6.05%), hexadecane (4.13%), heptadecane (3.27%), octadecane (5.92%), nonadecane (8.94%), eicosane (6.55%), heneicosane (0.82%), docosane (7.43%), tetracosane (2.85%), hexacosane (4.73%), octacosane (5.08%) and squalene (1.97%) relative % hydrocarbons and sterols, in addition to cholesterol (23.64%) and stigmasterol (18.07%)[26].

Table 6. Unsaponifiable matter components (%) of *Nepeta cataria L.*

Compound	Relative %
n- Dodecane	3.95
2,6- Dimethylundecane	0.8
α-Tocopherol	5.3
n- Pentacosane	0.84
n- Hexacosane	10.16
n- Heptacosane	2.7
n- Octacosane	1.12
n- Nonacosane	6.83
n- Triacontane	1.91
n- Henetricontane	26
n- Dotriacontane	2.98
n- Triatriacontane	2.57
α- amyrine	0.14
Squalene	0.68
β- sitoserol	18.6
Stigmasterol	8.9
Campesterol	6.52

Identification of terpenoids and sterols of *Nepeta cataria* L.

The dry powder was successively extracted with petroleum ether (40-60 ºC), chloroform, ethyl acetate and ethanol. The petroleum ether extract was the most potent successive extract[24,25]; therefore it was investigated. Four major compounds 1, 2, 3 and 4 were isolated according to their order of elution from the column. Their spectral characters as compared with cited data proved them to be α- amyrine, ixoroside aglycone, β sitosterol and ursolic acid.

The successive ethanol extract was concentrated to dryness and washed with petroleum ether. The residue was dissolved in least amount of dilute alcohol and hydrolyzed with H_2SO_4. The hydrolysate was extracted with petroleum ether. The petroleum ether extract was concentrated and tested on TLC, it gave two spots corresponding to β- sitosterol and ixoroside aglycone. The left aqueous solution was neutralized and chromatographed for sugars by paper chromatography where only glucose was detected. Consequently it was deduced that β- sitosterol and ixoroside are present as free aglycone in petroleum ether successive extract and are present in glucosidic form in the ethanol successive extract and glucose was the present sugar.

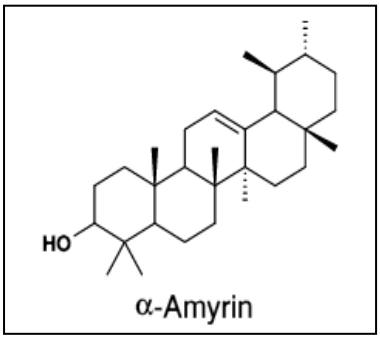


Fig.1. Structure of α-amyrin

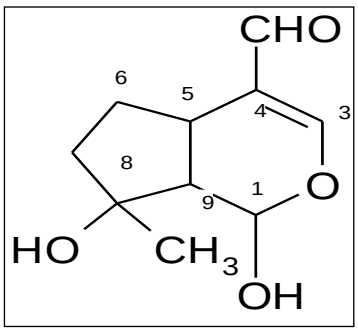


Fig.2 Ixoroside aglycone

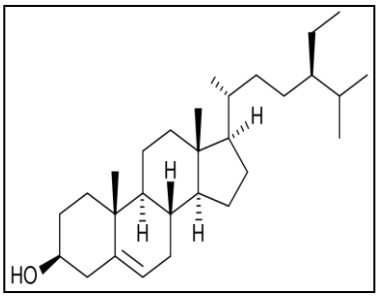


Fig.3 β- sitosterol

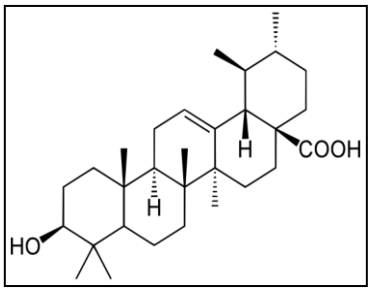


Fig.4 Ursolic acid

Compound 1 : (α-amyrin $C_{30}H_{50}O$ eluted by petroleum ether: Chloroform (70:30) Rf 0.76 (benzene: ethyl acetate (90:10), (+) Liebermann-Burchard test for terpenoids and steroids, **IR** thin film on KBr cm^{-1}; 3779, 3429 (OH), 2919, 2851 (CH, CH_2, CH_3), 1594 (C=C), 1466, 1422 (CH_3), 1118 (- OH), **MS** m/z; 391 (100%) base peak, 278.8 (70.6%), M^+ at 425 (M^+ -1) (60.7%), 378.7(32.4%), 230(35.3%), 316.7 (26.5%), 261.7 (23.5%), 213 (20.6%), 198.8 (17.6%), 157.8 (11.8%), 95.7 (10.3%) and 133.8 (7.4%), **^{1}H NMR** at (500 MHz , CDCl3) δ_H; 5.35 (H-12), 3.2 (H-3), 2.12 (H-18), 2.7 (H-22), 1.24 (Me-23), 0.87 (Me-30), 0.88 (Me-29), 0.89 (Me-24), 0.83 (Me-25), 1.24 (Me-26), 1.27 (Me-27) and **^{13}C NMR spectrum** δc (C-1) 38.8, (C – 2) 28.06 ; (C-3) 77.3; (C-4) 38.7; (C-5) 56.9; (C-6) 19.8; (C-7) 34.4; (C-8) 39.4; (C-9) 50; (C-10) 37.5; (C-11) 18.8; (C-12) 122; (C-13) 133; (C-14) 42.1(C-15) 29.3; (C-16) 28.9; (C-17) 51; (C-18) 56; (C-19) 40; (C-20) 40.2; (C-21)31.9; (C-22) 37.3; (C-23) 30.1; (C-24) 17.2; (C-25) 16.8; (C-26) 18.4; (C-27) 24.7; (C-28) 18.44; (C-29) 25 and (C-30) 22.7.The above data as compared with reported data[12,28] on *Nepeta cataria* supported substance (1) to be α-amyrin.

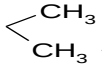
Compound 2 (Ixoroside aglycone $C_{10}H_{14}O_4$) eluted by petroleum ether: chloroform (60: 40) Rf 0.73, benzene: ethyl acetate (90:10). It was present as free aglycone as well as glucosidic form in successive ethanol extract and total ethanol extract upon acid hydrolyside of successive ethanol extract and extracting the aglycone part with petroleum ether and testing it on TLC, two spots were given, one corresponding to β- sitosterol and the other corresponding to Ixoroside aglycone. Aqueous solution was tested on paper chromatography for sugars (butanol: acetic acid: water (B: A: W) (4:1:5), it was formed to be glucose, **IR** thin film on KBr cm^{-1} 3430 (OH), 2922, 2854 (CH, CH_2, CH_3), 2667 (CHO), 1727 (CO), 1633 (C=C), 1464 (CH_3), 1255 (OH), **MS** Ixoroside and its aglycone M^+ at m/z 360 glucoside, base peak $C_{16}H_{24}O_9$ the aglycone M^+ at m/z 197 (360 – glucose) for $C_{10}H_{14}O_4$ (100%), m/z 167 (197-(H+CHO)) (37.4%), 201 (48.6%), 281 (42.9%) and 303 (31.4%) and **HNMR and ^{13}C NMR spectrum are listed in Table (7)**

Ixoroside was isolated for the first time from *Nepeta catria* either as glucoside or aglycone. It was reported in *Nepeta heliotropifolia* as glucoside only[28]**.**

Table 7. The HNMR and ^{13}C NMR spectrum of Ixoroside compound

C/H	δ_C	δ_H
A glycone		
1	99	5.3
3	159.4	7.4
4	122.3	-
5	29.1	2.16
6	28.07	2.14,1.12
7	39.46	1.61,1.24
8	77.6	-
9	51	2.3
10	24.9	1.07
11	191	9.3

Compound 3: β- sitosterol ($C_{29}H_{50}O$) eluted by petroleum ether: chloroform (60:40), Rf 0.55, benzene: ethyl acetate (90:10),**IR** thin film on KBr cm^{-1}**:** 3433 (OH), 2958, 2929, 2852 (CH, CH_2, CH_3)1464, 1380(gem dimethyl) 1628 (C=C), 1247 (-O-H), **MS** (M^+-2) at m/z 412 (41%) base peak 395 (M^+ -OH) (100%), 341 (91.2%), 300 (88%), 374 (55.9%) and 188 (51.5%), **^{1}HNMR** (500 MHz, CDCl3) δ : 4.12 (H-5), 5.3 (H-6), 3.52 (H-3), 2.28 (2H-4), 2.17 (2H-2), 1.25 (H-7), 1.01 (2H-1), 0.53 (M-18), 0.68 (M-19) and **^{13}C NMR** δ 36.58 (C-2), 72 (C-3), 37.3 (C-4), 121.8 (C-6), 11.9 (C-18), 19.1 (C-19), 18.8 (C-21), 19.4 (C-26), 12 (C-29). β- sitosterol in free and glucosidic reported in *Nepeta cataria L*., variety citriodral[12]. All the above findings as compared with cited data[12,28] supported that substance 3 is β- sitosterol.

Compound 4 : Ursolic acid ($C_{30}H_{48}O$) eluted by chloroform 100 %, Rf 0.42, positive (+) Liebermann-Burchard test for terpenoids and / or sterols (benzene: ethyl acetate (90:10), **IR** thin film on KBr cm^{-1}, 3428 (OH), 2917, 2850 (CH,CH_2,CH_3), 1709 (COOH), 1466 (C=C), 1410 (CH_3), 1298, 1274 () and 1147 (-O - H), M^+ at 453 (M^+-3) and base peak as well. m/z 408 (453- COOH) (64.3%), peak at m/z 391 (453- COOH-OH) (100%), 278.9 (55.7%), 233.5 (22.9%), 227 (18.6%), 158 (14.3%) and 95.3 (8.6%),**^{1}H NMR** (500 MHz , CDCl3) δ_H ; 5.35 (H-12), 3.2 (H-3), 2.216 (H-18), 1.2, 2.2 (H-22 a&b), 1.24 (Me-23), 0.87 (Me-30), 0.88 (Me-29), 0.99 (Me-24), 0.82 (Me-25), 1.2 (Me-26), 1.27 (Me-27) and **^{13}C NMR** (C-1) 38.7, (C – 2) 27.2 ; (C-3) 77.3; (C-4) 38.7; (C-5) 56.9; (C-6) 19.8; (C-7) 34.4; (C-8) 39.4; (C-9) 50; (C-10) 37.5; (C-11) 18.8; (C-12) 122; (C-13) 137; (C-14) 42.1(C-15) 29.3; (C-16) 28.9; (C-17) 51; (C-18) 56; (C-19) 40; (C-20) 40.2; (C-21)31.9; (C-22) 37.3; (C-23) 30.1; (C-24) 17.2; (C-25) 16.8; (C-26) 18.4; (C-27) 24.7; (C-28) 179.38; (C-29) 25 and (C-30) 22.7.The above data as compared with reported data[12,28] on *Nepeta cataria* supported substance (4) to be Ursolic acid.

Conclusion

To the best of our Knowledge and believe this is the first reported isolation of these compounds from *Nepeta cataria* and this is the first reported isolation of Ixoroside aglycone in nature**.**

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