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Research Paper

Evaluation of the primary and secondary products of *Nepeta septemcrenata* Erenb

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ABSTRACT

Investigation of free and combined sugars of *Nepeta septemcrenata* Erenb using HPLC revealing the presence of 6 free sugars and 9 combined sugars, while the analysis of the amino acids revealed the presence of 13 free amino acids and 17 protein amino acids. Meanwhile GLC analysis of the unsaponifiable lipid fraction revealed the presence of 12 hydrocarbons in addition to cholesterol and stigmasterol, as well as fatty acids which revealed the presence of 7 saturated fatty acids and 5 unstaurated fatty acids. Investigation of the flavonoid and phenolic acid constituents revealed the presence of apigenin, kaempferol, ferulic acid, kampferol-3-O-rhamnoside, kampferol-3-O-glucoside, kampferol-3-o-rhamnoside-4`-O-glucosiode and kaempferol-3-rutinoside.

Keywords: *Nepeta septemcrenata*, Primary products, Secondary products.

INTRODUCTION

Nepeta (Lamiaceae) is a genus of about 250 species. The members of this group are known as catnip or catmint because of their famed effect on cats-*Nepeta* pleasantly stimulates cat' pheromonic receptors, typically resulting in a temporarily induced euphoric state. Catnip is also known to induce mild euphoria in humans (Evans, et al., 1996). The genus is native to Europe, Asia and Africa, with the highest species diversity in the Mediterranean region east to mainland China. It is common in North America (Funk&Wagnalls). Only one species of this genus was recorded in Egypt and this species (*Nepeta septemcrenata*) is endemic for Sinai (Tackholm, 1974).

When cats sense the bruised leaves or stems of catnip, they may rollover it, paw at it, chew it, lick it, leap about and purr, often salivating copiously. Some cats will also growl, meow, scratch and/or bite the hand that is holding. This reaction only lasts for

about ten minutes before the cat loses interest. It takes up to two hours for the cat to "reset" after which it can come back to the catnip and have the same response as before (cat facts, 2003).

MATERIALS AND METHODS

The fresh plants of *Nepeta septemcrenata* were collected from SaintKathrine (Wadi Cherage) identified by local Bedouin. They were air dried at lab-temperature till constant weight, then ground to fine dry powder and kept to be used for different plant analysis.

Investigation of the primary product:

***Preliminary phytochemical screening*:** This includes testing for volatile oil by steam distillation method, steroids, coumarins, flavonoids, phenolics compounds, alkaloids using Dragend-orrf's, Mayer's and Wagner's reagents, glycosids and/or carbohydrates and saponins According to Balbaa, et al., (1981).

Investigation of carbohydrates:

***Identification of free sugars*:** Chaplin and Kennedy, 1994. HPLC of free sugars by the method of Nagel, 1992.

Identification of combined sugars: as described by Chaplin and Kennedy, 1994 and HPLC according to Nagel, 1992.

Investigation of free and protein-amino acids: according to Pellet and Young (1980), using Amino Acid Analyzer.

Investigation of lipid: The unsaponifiable matter and fatty acids components were determined using GLC according to Nelson, et al. (1969), Farag, et al. (1986) and Eaton, (1989).

Investigation of the secondary products:

Investigation of flavonoid and phenolic compounds (Radwan and Hassan, 2006): About 1.2 kg of defatted dried powder of *Nepeta septemcrenata* was macerated with 80% ethanol. The combined aqueous alcoholic extracts were evaporated in vacuo 45°C. The residue (40 g) was dissolved in hot distilled water (400 ml) and left over night. The aqueous filtrate was extracted with successive portions of chloroform (3 x 300 ml), followed by ethyl acetate (3 x 300 ml) and finally with n-butanol (3 x 300 ml). The ethyl acetate fraction was subjected to preparative paper chromatography (3MM, BAW 4:1:5). The bands (R_f 0.88, 0.87 and 0.86) were cut and eluted separately by 90% methanol. The butanol fraction was applied on the top of a glass column (110 x 6 cm) packed with polyamide in water-eluted was carried out using water followed by water/methanol mixtures and each fraction was 150 ml. The obtained combined fraction 9-25, eluted by H_2O/MeOH (80:20) was further purified using preparative PC (Whatman 3MM, BAW 4:1:5). The flavonoidal zones (R_f 0.82, 0.45 and 0.35 in BAW) was cut and eluted with 90% methanol. The combined fraction 32-40 was eluted by H_2O/MeOH (60:40) was further purified using preparative PC (Whatman 3MM, BAW). The flavonoidal zone (R_f 0.33 in BAW) was cut and eluted with 90% methanol.

RESULTS

Investigation of the primary products:

***Preliminary phytochemical screening*:** The plant contained volatile oil, steroids, terpenoids, flavonoids and phenolics and glycosides and/or carbohydrates. Neither coumarins, alkaloids nor saponins.

Investigation of carbohydrates:

Free sugars: The separation of the free sugars extract of *Nepeta septemcrenata* were achieved using high pressure liquid chromatography (HPLC), where the following sugars; arabinose, sorbose, galactose, glucose, rhamnose and raffinose were detected (Table 1).

***Combined sugars*:** Separation of the hydrolyzed combined sugars of *Nepeta septemcrenata* were achieved using HPLC, where it was found that the plant contained xylose, sorbose, galactose, glucose, rhamnose, raffinose, ribose, maltose and sucrose.

Investigation of amino acids;

***Free amino acids*:** The separations of free amino acids of *Nepeta septemcrenata* were achieved using amino acid analyzer. The relative percentage of each component was calculated and tabulated at table (1) thirteen free amino acids: aspartic acid, threonine, serine, glutamic acid, glycine, cysteine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine. The obtained results clarified that the concentration of histidine was the highest one (16.12 %) while the concentration of leucine represented the lowest one (2.07%).

***Protein amino acids*:** The investigation of hydrolyzed protein-amino acids of *Nepeta septemcrenata* was achieved using amino acid analyzer, where the relative percentage of each component were calculated and presented at table (1). The obtained results revealed that there were seventeen amino acids of different types and range of concentrations were detected. It was observed that the concentration of histidine was the highest one (12.54%) while the concentration of serine represented the lowest one (2.01%).

Investigation of lipids:

***Unsaponifiable matter content (hydrocarbons and sterols)*:** The unsaponifiable matter content of *Nepeta septemcrenata* was determined using GLC technique, where the relative percentage of each component was calculated and tabulated in table-2. The obtained results revealed that the plant contained twelve hydrocarbons, beside cholesterol and stigmasterol.

***Sapnifiable matter content (Fatty acids)*:** The fatty acids content of *Nepeta septemcrenata* were determined using GLC technique. The relative percentage of each component was calculated and tabulated in table-2. The obtained results revealed that the saturated fatty acids caprilic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid beside the unsaturated fatty acids myristoleic acid, palmitoleic acid, oleic acid, linoleic acid and arachidonic acid were present in *Nepeta septemcrenata* with different range of concentrations. It was clear from table-2, that stearic acid (20.08%) resembled the higher percentage of fatty acids followed by palmitic acid (18.75%) and that the lowest one was arachidic acid with the percentage of 0.74%.

Investigation of the secondary products:

***Investigation of flavonoid and phenolic acid compounds*:**

***Apigenin*:** R_f 0.88; isolated from the ethyl acetate fraction by preparative PC. (3MM, BAW) and eluted by 90% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.1) corresponding to that of apigenin (11 mg) which was identified by PC, UV (Table 3) and El-mass [M^+ 270 and fragments at m/z 242, 153, 121 and 118]. Further confirmation was preformed by carring out ^{1}H-NMR IN DMSO-d6 and the results were agreement with the reported data of apigenin (Mabry, et al., 1970).

Kaempferol: R_f 0.87; isolated from the ethyl acetate fraction by preparative PC. (3MM, BAW) and eluted by 90% methanol, gave after purification on Sephadex LH-20 column using 90% methanol asingle flavonoidal compound (no.2) corresponding to that of kaempferol (13 mg) which identified by PC, UV (Table 3) and Ms [M^+ 286 and fragments at m/z 285, 258, 229,121 and 93]. Further confirmation was performed by carring out ^{1}H-NMR in DMSO-d_6 and the results were agreement with the reported data of kaempferol (Mabry, et al., 1970).

***Ferulic acid*:** R_f 0.86; isolated from the ethyl acetate fraction by preparative PC. (3MM, BAW) and eluted by 90% methanol, gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.3) corresponding that of ferulic acid (15 mg) which identified by PC, UV (Table 3) and MS [M^+ 194 and fragments at m/z 179 and 77].

***Kaempferol-3-O-rhamnoside*:** R_f 0.82; isolated from the polyamide column of the butanol fraction (fraction 9-14) gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.4) Corresponding to that of kaempferol-3-O-rhamnoside which identified by PC, UV (Table 3) and Ms [M^+ 286 and fragments at m/z 285 (M^+-1) and 258 (M^+-Co), which are characteristic for that of kaempferol (Mabry, et al., 1970). The sugar moiety was identified as rhamnose [PC. Whatman No.1, ethyl acetate-pyridine-water (12:5:4) and n-butanol-benzene-pyridine-water (5:1:3:3)].

***Kaempferol-3-O-glucoside*:** R_f 0.45; isolated from the polyamide column of the butanol fraction (fraction 16-20) gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no.5) corresponding to that of kaempferol-3-O-glucoside which identified by PC, UV (Table 3) and Ms [M^+449]. Kaempferol (which identified by PC, UV (Mabry et al., 1970 Ms [M+ 286 and fragments at m/z 285 (M^+-1) and 258(M^+-1), which are characteristic for that kaempferol (Mabry, et al., 1970). The sugar moiety was identified as glucose [PC. Whatman No.1 ethyl acetate-pyridine-water (12:5:4) and n-butanol-benzene-pyridine-water (5:1:3:3)].

***Kaempferol-3-O-rhamnoside-4`-O-glucoside*:** $R_f$ 0.45; isolated from the polyamide column of the butanol fraction (fraction 21-25), gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no. 6) corresponding to kaempferol-3-O-ramnoside-4`-O-glucoside. UV spectrum with the different diagnostic shift reagents according to (Mabry *et al*., 1970), Table (3) showed typical MeOH and NaOMe UV spectra of kaemoferol, suggesting a 3-substituted flavonol. This evidence was supported by $AlCl_3$ and $AlCl_3$/HCl UV spectrum. ^{1}H-NMR in DMSO showed that signals corresponding to kaempferol (Mabry 1970) beside the sugar moiety at signals δ 4.9 (1H,d,J=2.5 Hz, H-1`` glucose), 4.1 (1H, broad, J=2.5Hz, H-1``` rhamnose), CH_3

rhamnose (d, broad 0.8) and 3.5 (m, remaining sugar protons). Acid hydrolysis (2NHCl) gave kaempferol which identified by PC, UV (Mabry, et al., 1970), the sugar moiety was identified as glucose and rhamnose [PC. Whatman No.1, ethyl acetate-pyridibe-water (12:5:4) and n-butanol-benzene-pyridine water (5:1:3:3)].

***Kaempferol-3-rutinoside*:** R_f 0.33; isolated from the polyamide column of the butanol fraction (fraction 32-38), gave after purification on Sephadex LH-20 column using 90% methanol a single flavonoidal compound (no. 7) corresponding to kaempferol-3-rutinoside. UV spectrum with the different diagnostic reagent according to (Mabry, et al., 1970), Table-3 showed typical MeOH and NaOMe UV spectra of kaempferol, suggesting a 3-substituted flavonol. ^{1}H-NMR in DMSO showed that signals corresponding to kaempferol (Mabry, et al.,1970) beside the signals characteristic for rutinoside sugar at δ 4.4 (1H, H-1``` rhamnose), δ 5.4(1H, anomeric proton, d, J=7Hz, H-1`` glucose), δ 3-4 (m, remaining sugar protons) and δ 0.9 (3H,d, J=6Hz, CH_3 rhamnose). Also acid hydrolysis gave kaempferol as the aglycone, glucose and rhamnose as the sugar moieties.

DISCUSSION

The separation of cholesterol from *Nepeta septemcrenata* was known as sterol occurring widely in animal tissues and some higher plants and algae (Matrin, 1985). Trease and Evans (1983) discussed also that cholesterol was widely distributed in plants. The results of the presence of higher percentage of stearic fatty acid followed by palmitic and Linoleic acids were in agreement with that of Goss (1973), who stated that the most abundant fatty acids of desert plant were palmitic, stearic and linoleic acid. The obtained results showed that *Nepeta septemcrenata* contained apigenin,which were in agreement with the presence of apigenin in *Nepeta cataria* L. var citriodora, (Modnicki, et al., 2007). On other hand *Nepeta cataria* contained luteolin 7-O-glucuronide, luteolin 7-O-glucurono-(1-->6)-glucoside, apigenin 7-O-glucuronide as well as free aglycones luteolin which were undetected in *Nepeta septemcrenata*, where it contrary contained flavonoids kaempferol, kaempferol-3-O-rhamnoside, kaempferol-3-O-glucoside , kaempferol-3-O-rhamnoside-4`-O-glucoside, kaempferol-3-rutinoside, beside the phenolic acid ferulic acid.

The presence of luteolin, apigenin and their glycosides, caffeic acid suggests the possibility of using lemon catnip herb as a constituent with mild sedative, antispasmodic, antioxidative and antiinflammatory action (Modnicki, et al., 2007). On other hand, Hertog, et al., 1993 stated that kaempferol and quercetine are flavonoids in regulary consumed foods may reduce the risk dead.

Meanwhile, Lazarus and Schmitz (2000) reported that dietary flavonoids may make an important contribution to cardiovascular health. Epidemiological studies have shown that intake of flavonoids may be inversely associated with long-term mortality from coronary heart disease in epidemiological studies. In the future, the authors believe that flavonoids could be the basis of new pharmaceuticals targeted to improve cardiovascular health. Brusselmans, et al. 2005, declared that the consumption of food products containing high amounts of flavonoids has been reported to lower the risk of various cancers. On other hand luteolin, quercetin, kaempferol, apigenin, and taxifolin, also markedly inhibited cancer cell lipogenesis.

They mentioned also that these compounds display very low toxicity for humans. Daily intake of relatively high doses of quercetin (1 g) or luteolin/apigenin (140 mg) for several weeks did not induce any side effects.

Hence the flavonoid and phenolic acid constituents of *Nepeta septemcrenata* declared the economic values of the plant as new source for human health.

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Table-1: Sugars and amino acids of *Nepeta septemcrenata* using HPLC and amino acid analyzer; tivelyrespec.

Sugars	Relative % of sugars		Amino Acids	Relative % of amino acids	
	Free	Combined		Free	Protein
Arabinose	11.54	-	Aspartic acid	6.02	4.86
Xylose	-	8.11	Threonine	10.08	2.27
Sorbose	9.47	10.56	Serine	5.01	2.01
Galactose	12.35	16.23	Glutamic acid	15.23	7.57
Glucose	30.15	21.04	Proline	-	9.83
Unknown	9.61	-	Glycine	7.02	5.28
Rhamnose	14.27	10.43	Alanine	-	4.96
Raffinose	12.05	9.47	Cysteine	2.34	2.28
Ribose	-	5.13	Valine	-	7.84
Maltose	-	7.08	Methionine	-	6.21
Sucrose	-	11.54	Isoleucine	6.15	8.35
			Leucine	2.07	4.03
			Tyrosine	14.21	8.81
			Phenylalanine	7.13	3.92
			Histidine	16.12	12.54
			Lysine	3.08	5.56
			Arginine	5.14	3.07

Table-2: G.L.C. of hydrocarbons, sterols and fatty acids of *Nepeta septemcrenata.*

Hydrocarbons and sterols			Fatty acids		
Hydrocarbons and sterols	No. of carbon atom	Relative %	Fatty acids	No. of carbon atom	Relative %
Dodecane	12	6.05	Caprilic acid	8:0	2.31
Hexadecane	16	4.13	Capric acid	10:0	3.92
Heptadecane	17	3.27	Lauric acid	12:0	5.84
Octadecane	18	5.92	Myristic acid	14:0	9.26
Nonadecane	19	8.94	Myristoleic acid	14:1	1.12
Eicosane	20	6.55	Palmitic acid	16:0	18.75
Heneicosane	21	0.82	Palmitoleic acid	16:1	10.52
Docosane	22	7.43	Stearic acid	18:0	20.08
Ttracosane	24	2.85	Oleic acid	18:1	11.26
Hexacosane	26	4.73	Linoleic acid	18:2	14.37
Octacosane	28	5.08	Arachidic acid	20:0	0.74
Squalene	29	1.97	Arachidonic acid	20:4	1.65
Cholestrol	27	23.64			
Stigmasterol	27	18.07			

Table-3: Ultra-violet spectral data (nm) of the isolated flavonoids.

Addition to MeOH flavonoides	**None**	**NaOMe**	**NaOAc**	**NaOAc + H_3BO_3**	**$AlCl_3$**	**$AlCl_3$ + HCl**
Compound (1)	266,296(sh), 337	274,325,392	274,300,377	268,301,339	276,301,347, 385	276,300, 340, 382
Compound (2)	253(sh),268, 342(sh),367	380,318,421	257,302(sh), 385	267,296(sh), 320(sh),370	266,305(sh), 350,422	266,305(sh), 350,422
Compound (3)	286,313	250(sh),291, 319				
Compound (4)	270,315,364	284,370,424	280,315,370	270,315,372	272,310,406	272,310,406
Compound (5)	264,296(sh), 350	274,324,400	274,302,372	269,300(sh), 350	302,346,398	276,302,344, 396
Compound (6)	265,328	265,390	272,385	272,328	295,375	295,375
Compound (7)	267,320, 353	272,320,400	270,310,382	260,370	266,355,422	266,298,422

