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ORIGINAL PAPER

Contributions to the Pharmacognostical Study on *Anethum graveolens*, Dill (*Apiaceae*)

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Abstract

The objectives of this study were the comparative pharmacognostical analysis of flowers, fruits and leaves of dill.

Microscopic examination showed the following specific anatomical elements: ovoids pollen grains, druses of calcium oxalate, fatty oil, aleurone, stomata of the diacytic and anomoytic type. The chemical analysis established the presence of flavonoids (rutin, quercetin), hydroxicinnamic acid derivates (caffeic acid, chlorogenic acid), coumarins (scopoletin), sterols (beta-sitosterol/stigmasterol) and mucilages. Flowers and leaves of dill had a higher amount of polyphenols comparative to fruits.

The results of the conducted study emphasized the fruits and flowers of dill as potential sources of essential oil.

Keywords: *Anethum graveolens*, polyphenols, essential oil

Introduction

Dill (*Anethum graveolens* L., *Apiaceae*) is a plant species often cultivated in Romania for the flavoring and curative properties (digestive disturbances accompanied by meteorism, flatulence and gastro-intestinal spasms, urinary infections, insomnia, galactogenical hyposecretion, etc) (C. Pârvu) [5]. The performed experimental studies demonstrated the antimicrobial, stomachic, antioxidant, carminative properties of dill (L.Jirovetz et al, M. Monsefi et al, M.Stavri & S.Gibbons, G.Q.Zheng et al) [3, 4, 8, 10].

The purpose of our researches was the pharmacognostical study of dill (flowers, fruits and leaves), cultivated in Romania, in order to establish quality criteria of this plant.

Materials and Methods

The raw material consists of flowers (*Anethi flores*), fruits (*Anethi fructus*) and leaves (*Anethi folium*), naturally dried after harvested from *Anethum graveolens* cultivated in Romania.

For microscopic study clarified preparations were used with a chloral hydrate solution 800 g/L (Zeiss microscope; ob. 10x and 40x). For the qualitative analysis the raw material was successively extracted with different solvents (ethyl ether, methanol, water). Half of the above alcoholic and aqueous solutions were hydrolyzed. Specific reactions were carried out in initial and hydrolyzed solutions, in view to identify the active principles (C.E.Gîrd, L.E.Duțu) [1, 2]. For polyphenols (flavonoids and hydroxicinnamic acid derivates), coumarins, and

sterols/triterpenes was applied thin-layer chromatography (TLC) (M.L. Popescu, M. Dinu) [6, 7, 11].

Parameters of TLC for polyphenols (flavonoids and hydroxicinnamic acid derivates) (M.L. Popescu, M. Dinu) [6, 7]

Test solution: 1g powdered of these products was extracted with 10 mL methanol R for 10–15 min., filtered and concentrated; TLC plates silica gel F Merck; solvent system (mobile phase) ethyl acetate : conc. formic acid : water/ 80:8:12 (V/V/V); reference solution (0.1% in methanol): rutin (Fluka), quercetin (Merck), caffeic acid (Merck), chlorogenic acid (Merck); detection: spraying with methanolic solution 1% of diphenylboriloxietilamine and methanolic solution 5% of propylene glycol 400 (successively sprayed) and UV light (366 nm).

Parameters of TLC for coumarins

Test solution: 1g powdered of these products was extracted with 10 mL methanol R for 10–15 min., filtered and concentrated; TLC plates silica gel F Merck; solvent system (mobile phase): conc. formic acid : water : methanol : ethyl acetate/ 2,5:4:4:40 (V/V/V/V); reference solution (0.1% in methanol): scopoletin (Merck); detection: spraying with methanolic solution 1% of diphenylboriloxietilamine and UV light (366 nm).

Parameters of TLC for sterols / triterpenes (M.L. Popescu, M. Dinu) [6, 7]

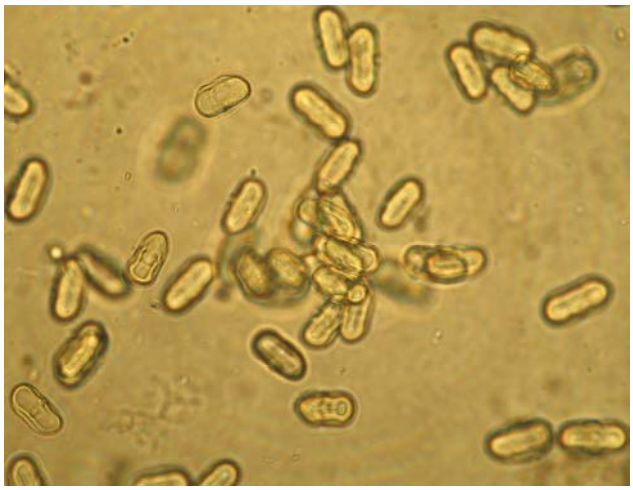
Test solution: 1g powdered of these products was extracted with 10 mL chloroform R for 10–15 min., filtered and concentrated; TLC plates silica gel F Merck; solvent system (mobile phase) chloroform : acetone / 8:2 (V/V); reference substances (Sigma; 0,1% in methanol): ursolic acid, oleanolic acid, beta-sitosterol, stigmasterol; detection: spraying with acetic anhydride and ethanolic sulphuric acid (1:1), heating at 100ºC for 10 min. and UV light - 366 nm (using a Camag UV lamp).

In order to evaluate the quality of the herbal product, the loss on drying process was determined (according to European Pharmacopoeia 5-th edition), flavonoids (using a spectrophotometrical method based on the reaction with aluminium chloride, according to Romanian Pharmacopoeia 10-th edition, *Cynarae folium* monograph, etalon curve of rutin) and total hydroxycinnamic acid derivatives (according to European Pharmacopoeia 5-th edition, Ash leaf monograph, using a spectrophotometrical method based on the reaction with sodium nitrite and sodium molybdate [11, 12]. The spectrophotometric determinations have been carried out using a spectrophotometer *Cecil series 2000*.

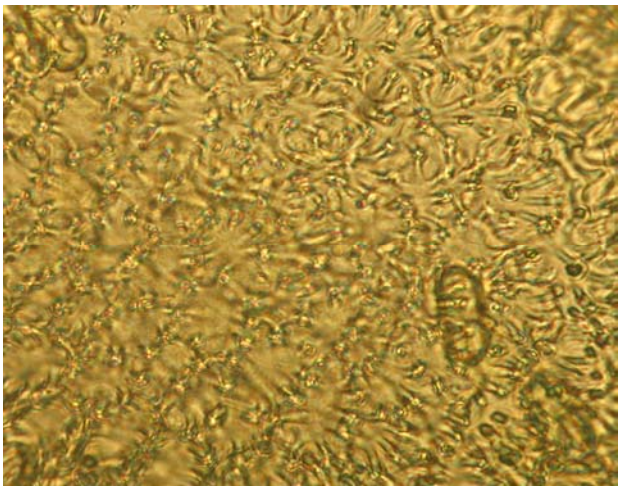
Results and Discussion

The macroscopic examination established the morphological characteristics and confirmed the identity of the raw material (based on the correspondence of the morphological characters of raw material with these described by scientific data) (C. Pârvu, E. Teuscher et al) [5, 9, 13].

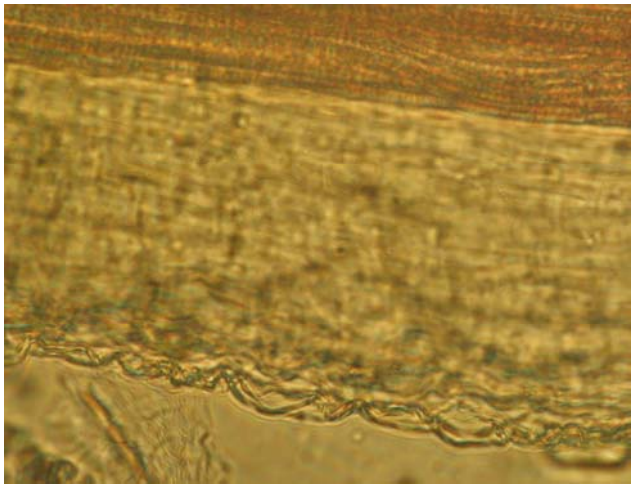
In the microscopic examination of *Anethum graveolens,* the following anatomical elements were observed (fig. 1): ovoids pollen grains with 2 pores and smooth exine, endothecium, papillae, aleurone grains in *Anethi flores*; fragments of the endosperm and cotyledons containing fatty oil, druses of calcium oxalate, cellulose fibers, pigmented cells, lignified vessels in *Anethi fructus*; parenchyma of the mesophyll with small vessels, fragments of epidermis with stomata of the diacytic and anomoytic type in *Anethi folium*.



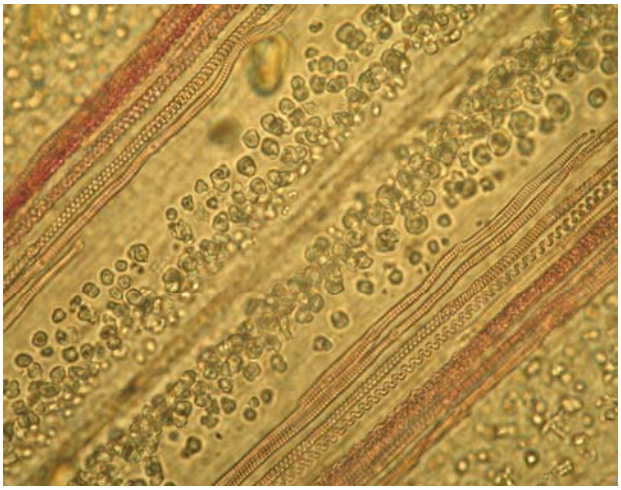
A. pollen



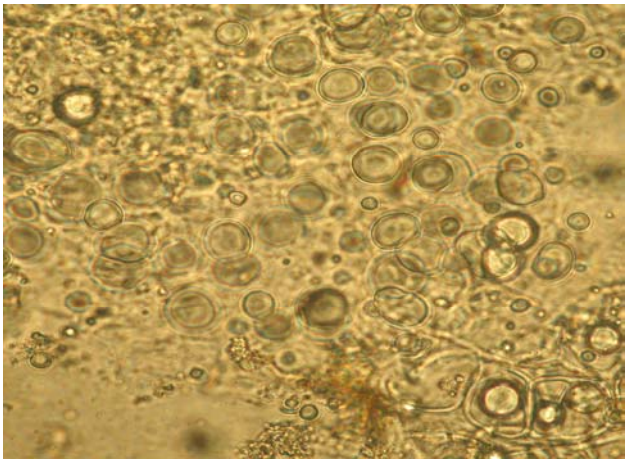
B. endothecium



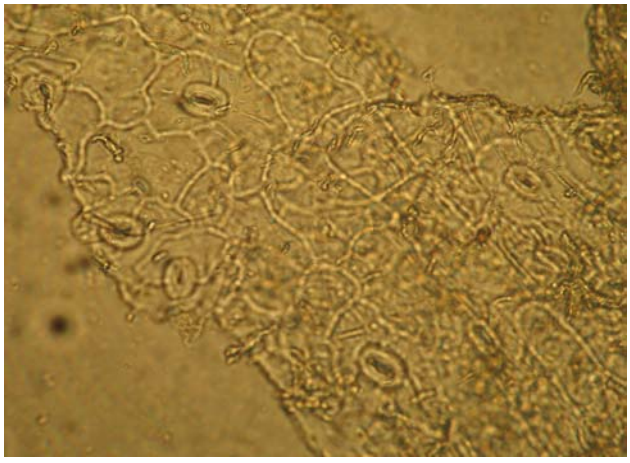
C. papillae



D. small vessels, aleurone



E. oil droplets



F. stomata

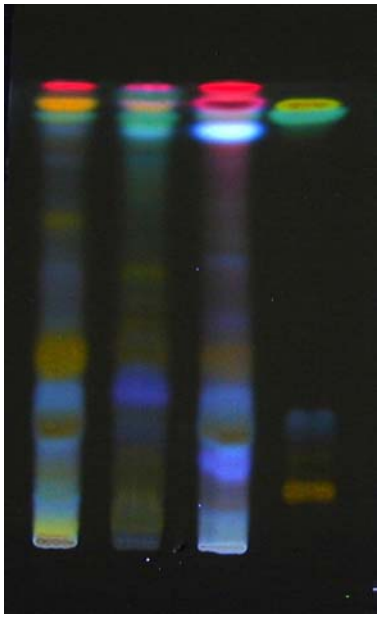
Figure 1. Microphotographs showing anatomical elements of *Anethum graveolens*

We consider that the specific anatomical elements for the *Anethum graveolens* species are represented by: aspect of pollen grains, crystallization state of calcium oxalate (druses),

the nature of stock substance (fatty oil, aleurone) and type of stomata (diacytic and anomoytic).

Flavonoids (aglycones and glycosides), hydroxycinnamic acid derivatives, coumarins, sterols/ triterpenes, and mucilages were identified in these products by specific chemical reactions. These compounds are mentioned in the scientific data about *Anethum graveolens*. The presence of sterols/ triterpenes was not specified by scientific sources that had consulted (C. Pârvu, E. Teuscher et al) [5, 9, 13].

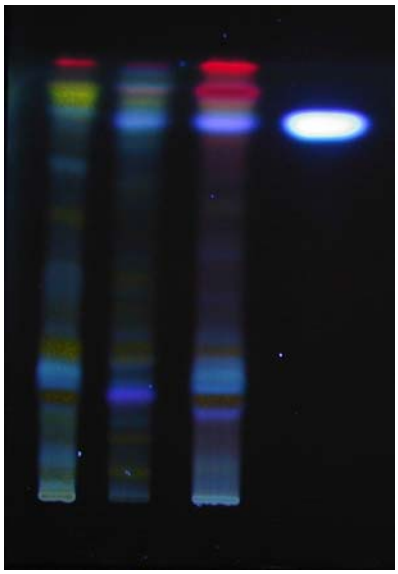
By analyzing TLC chromatogram of polyphenols (fig. 2) we can note the presence of several spots corresponding to compounds with flavonoid behavior (yellow or yellow-brown fluorescensce, after spraying with diphenylboriloxietilamine) or de hydroxycinnamic acid derivatives (blue or green-blue fluorescensce, after spraying with the mentioned reagent). Among these, rutin (Rf = 0,24), caffeic acid (Rf =0,32), chlorogenic acid (Rf =0,91) and quercetin (Rf = 0,92) have been identified in all the analyzed plant products. The consulted scientific data mentions flavonoids (glycosides of quercetin and isorhamnetin in *Anethi herba*, glycosides of kempferol in *Anethi fructus*) (C. Pârvu, E. Teuscher et al) [5, 9, 13].



A- flowers extract
B- fruits extract
C- leaves extract
D- reference substances (from top downwards: quercetin, caffeic acid, chlorogenic acid and rutin)

Figure 2. TLC chromatogram of polyphenols in methanolic extracts prepared from *Anethum graveolens* flowers, fruits and leaves (viewed with a UV lamp at 366 nm).

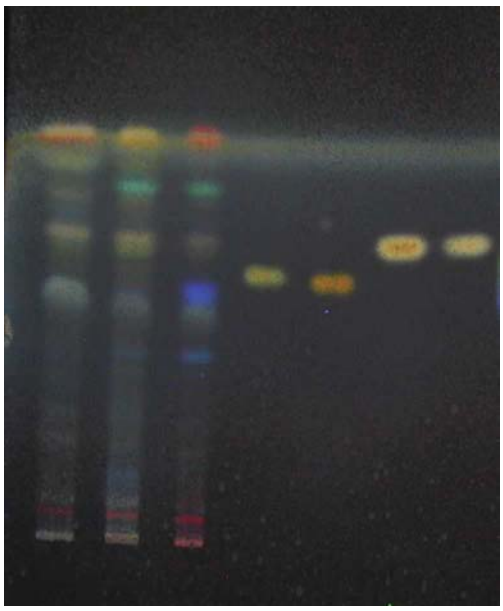
The analysis of TLC chromatogram of coumarins (fig. 3) shows the presence of several spots corresponding to coumarins (compounds with bleu fluorescence, deeper after spraying with diphenylboriloxietilamine). Scopoletin (Rf = 0,92) has been identified in all analyzed plant products. The scientific data notes the presence in *Anethi herba* of simple coumarins (scopoletin, coumarin), respectively of coumarins (umbelliferone) and furanocoumarins (bergapten, xanthotoxin) in *Anethi fructus* (C. Pârvu, E. Teuscher et al) [5, 9, 13].



A- flowers extract
B- fruits extract
C- leaves extract
D- reference substance (scopoletin)

Figure 3. TLC chromatogram of coumarins in methanolic extracts prepared from *Anethum graveolens* flowers, fruits and leaves (viewed with a UV lamp at 366 nm).

Beta-sitosterol/ stigmasterol (Rf=0.64, violet color in visible and yellow fluorescence in UV, after spraying with acetic anhydride and H_2SO_4/ethanol) was identified by TLC in all tree analyzed plant products (fig. 4). These compounds are classical examples of vegetal sterols. The two triterpene aglycones, used as reference substances, oleanolic acid (Rf=0,73) and ursolic acid (Rf=0,72) have not been identified, in these TLC conditions, in flowers, fruits and leaves of dill. In the scientific data there are no specifications about the presence of sterols/triterpens in *Anethum graveolens* (C. Pârvu, E. Teuscher et al) [5, 9, 13].



A- flowers extract
B- fruits extract
C- leaves extract
D- reference substances (oleanolic acid)
E- reference substances (ursolic acid)
F- reference substance (beta-sitosterol)
G- reference substance (stigmasitosterol)

Figure 4. TLC chromatogram of triterpenes in chlorohormic extracts prepared from *Anethum graveolens* flowers, fruits and leaves (viewed with a UV lamp at 366 nm).

The results of the quantitative chemical analysis (calculated with reference to the dried drug) are presented in the table 1.

Table 1. The results of quantitative chemical analysis

Parameter	Results		
	Anethi flores	***Anethi fructus***	***Anethi folium***
Swelling index	10,2 - 10,7	6,8 – 7,2	10,1 – 10,8
Flavonoids (g%, expressed as rutin)	0,226–0,243	0,022–0,025	0,196-0,218
Total hydroxycinnamic acid derivatives (g%, expressed as chlorogenic acid)	1,758–1,905	0,135-0,141	1,107–1,317
Volatile oil (mL/100 g raw material)	2,9 – 3,3	3,1 – 3,7	1,1-1,4

The results show that the swelling index has relatively high values, which can be correlated with the presence of water – soluble polysaccharides (mucilage).

Anethi flores and *Anethi folium* have an appreciable content of polyphenols (flavonoids and hydroxycinnamic acid derivatives). The results show that these values are lower than the ones mentioned in the scientific data for other plan products, characterized in the same experimental conditions. By instance, in case of the leaf of artichoke (*Cynarae folium*), FR X stipulates not less than 0,35 % flavonoids expressed as rutin. FE 5 stipulates for the ash leaf (*Fraxini folium*), not less than 2,5% hydroxicinnamic acid derivates, expressed as chlorogenic acid [11, 12].

Anethi fructus and *Anethi flores* can be considered as volatile (essentiale) oil sources. The scientific data mention that the pharmacological properties of some species of *Apiaceae* Family are due to the content of volatile oil. FR X stipulates for some products obtained from the species in this Family not less than: 2 % essential oil for *Anisi vulgaris fructus* (fruits of anise), 3 % essential oil for *Carvi fructus* (fruits of caraway), 3,5 % essential oil for *Foeniculi fructus* (fruits of fennel). *Anethi folium* has a small content of essential oil [12].

Conclusions

The pharmacognostical screening of flowers, fruits and leaves of dill has been achieved. The specific anatomical elements are pollen grains, druses of calcium oxalate, type of the stock substance (fatty oil, aleurone) and type of stomata. The main classes of active principles are flavonoids (quercetin, rutin), hydroxycinnamic acid derivatives (caffeic acid, chlorogenic acid), coumarins (scopoletin), sterols (beta-sitosterol / stigmasterol) and mucilage. *Anethi fructus* and *Anethi flores* can be considered essential oil sources.

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