

RESEARCH ON NIGELLA SATIVA L. (RANUNCULACEAE) MUCILAGE

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REZUMAT

Lucrarea de față constituie o analiză chimică asupra părților aeriene fără semințe ale speciei *Nigella sativa* (Ranunculaceae). Pentru prima dată în literatură a fost semnalată prezența mucilagiilor într-un procent de 4,8% în părțile aeriene și rădăcinile speciei *Nigella sativa* (Ranunculaceae). Cu ajutorul unui procedeu de cromatografie pe strat subțire cuplat cu fotodensitometrie au fost evidențiate componentele mucilagului, precum și procentele lor de combinare: acid galacturonic (26,08%), acidglucuronic (11,40%), galactoză (12,39%), glucoză (18,21%), arabinoză (20,03%), ramnoză (11,89%).

Cuvinte cheie: *nigella sativa*, ranunculaceae, mucilag, cromatografie pe strat subțire, fotodensitometrie

ABSTRACT

The paper presents the chemical analysis of *Nigella* vegetal species, of the aerial, seedless parts of the plant. It is pointed out for the first time the presence of 4.8% mucilages in the aerial part (herba) and root (radix) of *Nigella sativa* (Ranunculaceae). As a result of thin layer chromatography and photodensitometric evaluation of chromatograms, galacturonic acid (26.08%), glucuronic acid (11.40%), galactose (12.39%), glucose (18.21%), arabinose (20.03%) and rhamnose (11.89%) were identified and assayed as the components of mucilage.

Key Words: *Nigella sativa*, Ranunculaceae, mucilage, thin layer chromatography, photodensitometry

PREMISES

Nigella (Ranunculaceae) genus is used as natural remedy for more than 2000 years ago in the Far and Middle East countries.^{1,2}

A little bottle with oil of *Nigella sativa* seeds was discovered in the tomb of pharaoh Tutankamun, suggesting that *Nigella sativa* had a critical role in Egyptian ancient embalming practices.

Nigella sativa L. (Ranunculaceae), one of the worldwide spread species of *Nigella*, is an annual herbaceous plant. The common name of plant is fennel flower.^{3,4}

MATERIAL AND METHOD

The vegetal material used is formed by aerial part and root, gathered in July 2002 from a culture placed in Vladimirescu village, Arad county. Fresh product was laid out to dry at normal indoor temperature, then made small pieces of a sprayed. Soaking factor determination procedure was used for vegetal product *Nigellae fructus sine seminibus* as well.⁵

Vegetal produce, separated on morphological category, stem, leaves, flowers, and seedless fruits, was qualitatively and quantitatively analyzed.⁶

The determination of soaking factor was performed on the root, the aerial part and the fruit fine powder, according to the procedure described in the Romanian Pharmacopoeia Xth edition.⁴ For each morphological category, three measurements were done, the final result being the average of the three individual values obtained.^{7,8}

For quantitative determination (gravimetric analysis) of mucilage in *Nigellae sativae herba*, *Nigellae sativae radix*, the vegetal produce was successively extracted with white spirit, chloroform, and methanol

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in Soxhlet apparatus in order to remove extractible compounds with these solvents. Anhydrous powder obtained is subsequently extracted on boiling water bath for 30 minutes using an ascending distillate cooler with refluxing the solvent, followed by precipitation in solution of 1% ethanolic acetic acid. The precipitate obtained was separated by centrifugation, then dried in and weighted.¹

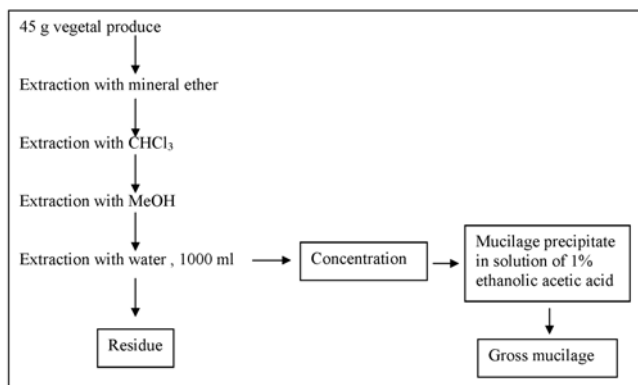


Figure 1. Mucilage separation sheet

Mucilage separated was purified by water dissolution and re-precipitation with acidified ethanol, dried and weight again. Mucilage content was referred to 100 g dry vegetal produce.⁸

The qualitative analysis of *Nigella sativa* mucilage implies hydrolysis of separated mucilage with 4% sulfuric acid for 90 minutes. The product of hydrolysis was neutralized with barium carbonate.^{2,7} The chromatographic separation was done on silicagel thin layer using several mobile phases, the best results being provided by the following experimental conditions: Kieselgel 60 F₂₅₄ Merck as stationary phase, n-butanol: acetic acid:water mixture (4:1:5) as mobile phase. During the migration time (1 h), the migration distance covered was 7 cm. The sample (10 ml) was linearly spotted at the start line of thin layer.

The standard solutions of glucose (1), galactose (2), glucuronic acid (5), galacturonic acid (4), arabinose (3), xylose (6), rhamnose (7), maltose (8) and fructose (9), each of them at concentration 1 % in methanol, were applied on thin layer. (Fig. 2)

As developing reagents aminohippuric acid and thymol-sulfuric acid were used. After application of developing reagents, the plate was heated at 110°C for 5 minutes.

As a result of chromatographic analysis of hydrolysis product, galacturonic acid, glucuronic acid, galactose, arabinose and rhamnose were identified. Identifications were based on the specific fluorescence at ultraviolet region of the reaction products of hexose,

pentose and uronic acid, formed after treatment with aminohippuric acid, and on the colorations appeared after treatment with thymol-sulfuric acid reagent (pink, violet, orange). The chromatograms developed with thymol-sulfuric acid reagent were evaluated quantitatively by photodensitometry at 500 nm with a Shimadzu CS-9000 apparatus.

RESULTS

Nigella sativa mucilages are solid amorphous, white-brown substances, soluble in cold water at pH 5.5.

Soaking factor values were variable as follows: leaves 9.5, fruits 11, stems 9, flowers 11, roots 8. It has been found that mucilage is variable, the highest content being noticed in fruits and flowers.

Mucilages are heteropolysaccharides of molecular weight between 5×10^4 and 5×10^6 , acid or neutral type depending on the nature of monomer (hexose, pentose, and uronic acid). Mucilage is divided into ozuronic (with uronic acid content) and neozuronic (without such acid) types.

Some authors have demonstrated the presence of some polysaccharides in *Nigellae* semen, but not in *Nigellae* herba.

According to quantitative analysis, the mucilage represents 4.8 % of the chemical components in the *Nigellae* herba product.

Chromatographic separation of components in the standard solutions and in the hydrolysis product is illustrated in Figure 2.

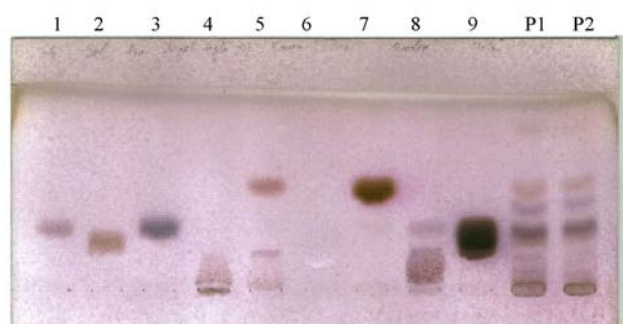


Figure 2. Thin layer chromatographic separation of glucides from *Nigella sativa* (Ranunculaceae) obtained after hydrolysis of mucilage
Legend: 1 - glucose; 2 - galactose; 3 - arabinose; 4 - galacturonic acid; 5 - glucuronic acid; 6 - xylose; 7 - rhamnose; 8 - maltose; 9 - fructose; A - sample 1; B - sample 2. Sample 1 - herba mucilage hydrolysate of *Nigella sativa*. Sample 2 - herba mucilage hydrolysate of *Nigella sativa*

The following components were identified and assayed in hydrolysate: galacturonic acid, glucuronic acid, galactose, glucose, arabinose and rhamnose. (Table 1, 2 and 3)

Table 1. Components identified by chromatography

No.	Monomer identified	Rf	Color after thymol-sulphuric acid revealing
1	Galacturonic acid	0.22	Violet
2	Glucuronic acid	0.26	Pink
3	Galactose	0.42	Pink
4	Glucose	0.50	Pink
5	Arabinose	0.58	Violet
6	Rhamnose	0.66	Orange

Table 2. Results after photodensitometric analysis

No.	Monomer identified	%
1	Galacturonic acid	26.08
2	Glucuronic acid	11.40
3	Galactose	12.39
4	Glucose	18.21
5	Arabinose	20.03
6	Rhamnose	11.89

Table 3. Constituent types in *Nigellae herba* mucilage

Constituent type	%
Azuronic acid	37.48
Simple ozes	62.52

DISCUSSION

According to analysis, soaking factor values are: 9.5 for leaves, 11 for seedless fruits, 9 for stem, 11 for flowers, 8 for roots. Values between 8-11 are characteristic for some products as *Altheae radix*, *Altheae folium*; the maximum mucilage content is 10 and the minimum content is 4 for *Lini semen*.³

Six components were identified after mucilage hydrolysis: galacturonic acid, glucuronic acid, galactose, glucose, arabinose and rhamnose.

By densitometry a galacturonic acid content of 26.08% has been found.

Presence of uronic acids in separated mucilage monomers means that mucilage belong to ozuronic mucilage class.

CONCLUSION

- This study pays attention for the first time to the existence of mucilage in different morphologic category of *Nigella sativa*.

- Average soaking factors have values from 8 (root) to 11 (flowers and seedless fruits).

- Mucilage separated from *Nigella sativa* is a solid, amorphous, brown colored, soluble in water.

- Glucides in mucilage composition are: galacturonic acid (26.08%), glucuronic acid (11.40%), galactose (12.39%), glucose (18.21%), arabinose (20.03%), and rhamnose (11.89%).

Mucilages separated from *Nigella sativa* are of ozuronic type.

REFERENCES

1. Beker G. *Plantes toxiques*. 1984 by Gründ Librairie, Paris, p.74
2. XXX - *Dying Reagents for Thin Layer Chromatography*. Merck (Eds.), Darmstadt, Germany, 1974;97.
3. Ciulei I, Grigorescu E, Stanescu U. *Plante medicinale, Fitochimie si Fitoterapie*. Vol I, Editura Medicala, Bucuresti, 1993.
4. Hoppe A. *Drogenkunde, Band I. Angiospermen* 8 Auflage, 1975; 345.
5. Popescu H et al. *Analiza chimică a produselor naturale medicinale*. Litografia UMF, Cluj-Napoca, 1981:1-6.
6. Baumgarten G. *Die herzwirksamen Glykosyde*. Georg Thieme Verlag, Leipzig, 1963;84-86.
7. *Farmacopeea Română editia a X-a*. Editura Medicală, București, 1993;1053-5, 1057-8.
8. Stahl E, Schild W. *Pharmazeutische Biologie Vol II*. Gustav Fischer Verlag, Stuttgart-New-York, 1981;308-17.
9. Gyeresi A. *Vademecum Cromatografic*. Editura Mentor, Bucuresti, 1997;175.