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CHEMICAL COMPOSITION OF *GLEDITSIA TRIACANTHOS* L. - APPLICATION IN PHYTOTHERAPY OF SOCIALLY SIGNIFICANT DISEASES

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Abstract

Isolation and identification of biologically active substances from medicinal plant Gleditsia triacanthos L. are examined. Development of methods for obtaining medical extracts of Gleditsia triacanthos L. is of practical importance in medicine for prevention of cardiovascular system diseases, antispasmodics and biliary clearance acting drugs as well as in medicinal cosmetics.

Key words*:* *Gleditsia tricanthos* L., *triacantine, fatty acids, phospholipids, sterols, tocopherols, polyphenols, HPLC, GC/MS*

1. INTRODUCTION

Fabaceae family is the third largest family of flowering plants after Orchidaceae and Asteraceae. This family is distributed throughout temperate and tropical regions of the world (Rundel, 1989). Among the Fabaceae is the genus Gleditsia, the Locust tree which comprises about 14 species of deciduous trees (Huxely et al., 1992), and is native to North America and Asia. Gleditsia species have been widely used in folk medicine. They are used for treatment of carbuncle, scabies, skin diseases and for treating apoplexy, headache, productive cough and asthma, also they are used as diuretic and expectorant (Miyase et al., 2010).

The seeds contain carbohydrates, fibers, starch, proteins, lipids, vitamins (A, B, K), considerably low content of glyceride oil (0.8 - 4.3%). The main components in triacylglicerols were found to be palmitic (34.4%), oleic (38.4%), stearic (16.5%) and linoleic acid (9.0%) (Mariod, 2008). It content of phospholipids in the oil was 0.32%, but has not information about individual composition of phospholipid fraction. In tocopherols fraction predominates mainly α-tocopherol. (Rakhmanberdyeva et al., 2002). It was not established data about sterol composition of the oil.

In the present investigation we have attempted to characterise the content and composition of glyceride oil, recovered from the seeds of *Gleditsia triacanthos*, cultivated in Bulgaria and the content and composition of sterols, phospholipids and tocopherols of the glyceride oil.

The alkaloids are a group of molecules with a relatively large occurrence in nature around the Globe. They are very diverse chemicals and biomolecules, but they are all secondary compounds and they are derived from amino acids or from the transamination process. The alkaloids are classified according to the amino acids that provide their nitrogen atom and part of their skeleton. Similar alkaloids can have quite different biosynthetic pathways and different bioimpacts. These alkaloids are derived from l-lysine, l-ornithine, l-tyrosine, l-tryptophan, l-histidine, l-phenylalanine, nicotinic acid, anthranilic acid or acetate. The terpenoid, steroid and purine alkaloids are also important. Millions of people over the World use purine alkaloids every day whether starting the day with a cup of coffee or drinking a cup of tea in the afternoon (Aniszewski, 2007).

Prior to 1900 there was a period in which a variety of alkaloidal and pharmacological activity was ascribed *to Gleditsia triacanthos* L. Other early reports suggested the presence of alkaloidal material in species of Gleditsia (Greshoff et al., 1900, Wehmer, 1929) and Belikov, Bankowsky and Tsarev (Belikov et al., 1954, Belikov et al., 1959) reported the isolation of the alkaloid triacanthine from the young leaves of *Gleditsia triacanthos*, to which they assigned the formula $C_8H_{10}N_4$. This empirical formula appeared intriguing due to the high ratio of nitrogen to carbon, which is in contrast to most other alkaloidal formulas, and investigation of triacanthine was especially attractive in view of the abundance of *Gleditsia triacanthos*. During 60's and 70's years of 20th century few researchers had isolated the alkaloid triacanthine from *Gleditsia triacanthos* L (Leonard, 1962, Morimoto et al., 1963, Panova et al., 1971).

The process of burning fat cells and in the extracellular space in the human body is supported from presence of the alkaloid triacanthine isolated from *Gleditsia triacanthos* L leaves. This effect is very suitable for the

development of medicinal products used for the various conditions associated with the accumulation of fats, as is the case with cellulite.

The antioxidants are natural compounds which have been studied extensively for decades in order to find compounds protecting against a number of diseases related to oxidative stress and free radicals. The plant kingdom offers a wide range of compounds exhibiting antioxidant activities. Polyphenolic compounds including flavonoids, phenolic acids and tannins have been considered as excellent natural antioxidants. They are widely distributed and can be considered as the most abundant plant secondary metabolites (Choudhary et al., 2013, Yin et al., 2008, Mazandarani et al., 2012). That is the reason for the overall study of the effectiveness of antioxidants from medicinal plants which are subject of active research. Some of the phenolic natural products such as flavonoids are of particular interest because of their antioxidant activity through scavenging oxygen radical. It has been reported that the ability of these compounds for scavenging the free radical, plays an important role in many diseases such as cardiovascular diseases, aging, cancer and inflammatory disorders (Anusuya et al., 2013, Cakir et al.,2006, Moresco et al., 2008). The stable 1,1-diphenyl picrylhydrazyl (DPPH•) free radical is model for detecting the ability of plant extracts and compounds for the free radical- scavenging activity in a relatively short time and it is simpler than the other assay models. This observation has accelerated the search for potential antioxidant principals from plants (Moresco et al., 2008, Zhou et al., 2007).

Previous reports on Gleditsia species discovered that they contain bioactive compounds as phenolic compounds; phenolic acids (Zhou et al., 2007, Santamour, 1977), flavonoids (Santamour, 1977, El-Sayed et al., 2013, Ragab et al., 2010, Zhang et al., 2011, Li et al., 2005). Mohamed and co-workers reported isolatation of eight flavonoids including six flavonoid glycosides vicenin-II, lucenin-I, isoorientin, orientin, vitexin, isovitexin together with two aglycones: luteolin and apigenin (Mohamed et al.,2013). Other researchers reported isolatation of glycosides of apigenin and luteolin: isovitexin, apigenin-7-*O*-*β*-*D*-glucopyranoside and luteolin-7-*O*-*β*-*D*-glucopyranoside (El-Sayed et al., 2013).

All of these biologically active compounds can be isolated and analyzed using chromatographic approaches. HPLC system could be used for identification of herbal extracts. The chromatographic profile HPLC gave is unique for each herb and served as his fingerprint. Therefore our study is related with analysis of *Gleditsia triacanthos* extract. In the present work is an attempt for determining the phenolic compounds and flavonoids, beside detecting the ability of the 70% methanol extract of the leaves of *Gleditsia triacanthos* L (Dagnon et al., 2013).

This research is subjected to isolation and identification of the alkaloid triacanthine from Gleditsia *triacanthos* L. leaves.

The aim of this study is to characterize, quantify content and composition of glyceride oil, recovered from the seeds and the polyphenols content in the leaves of *Gleditsia triacanthos.*

2. MATERIALS AND METHODS

All solvents and reagents were of analytical grade and were used without additional purification. Reference phospholipids and fatty acid methyl esters were purchased from Fluka (Chemie Gmbh, Switzerland). Reference tocopherol isomers and individual sterols were purchased from Merck (Darmstadt, Germany). Thin-layer chromatography (TLC) plates were prepared in the laboratory using Silica gel 60 G (Merck, Darmstadt, Germany). Besides we have attempted to characterise the content and composition of glyceride oil, recovered from the seeds of *Gleditsia triacanthos*, cultivated in Bulgaria and the content and composition of sterols, phospholipids and tocopherols of the glyceride oil.

All solvents used for extraction, including methanol (MeOH), dichloroethane (DCE), ammonia (NH_3; 25% w/w solution) and sulfuric acid (H_2SO_4; 98.08% w/w), were of analytical grade. The solvents were procured from Merck™ (Germany).

Methanol used for HPLC analyses were of chromatographic grade (VWR, Austria). Water for HPLC was prepared with Millipore purifier (Millipore, USA). Acetic acid HPLC grade, Merck, Germany were used to prepare the mobile phases. Neochlorogenic acid (Neo) (Cas Number 906-33-2), chlorogenic acid (ChA) (Cas Number 327-97-9), rutin (Rut) (Cas Number 250249-75-3), quercetin (Qrc) (Cas Number 117-39-5) luteolin (Lut) (Cas Number 491-70-3) and apigenin (Apg) (Cas Number 520-36-5) were purchased from Sigma Aldrich, HPLC standards, purity >95%. Standard solutions with concentrations from 0.1 up to 0.005 mg/ml were obtained by diluting the stock solution of each component in methanol.

Samples

The seeds and leaves of *Gleditsia triacanthos* were obtained in the region of southern Bulgaria crop 2013.

Isolation of glyceride oil and determination of oil content

The seeds (100 g sample) were air-dried and the oil was extracted with n-hexane in Soxhlet for 8 h. The solvent was partly removed in a rotary vacuum evaporator, the residue was transferred in pre-weight glass vessels and the rest of the solvent was removed under a stream of nitrogen to a constant weight to determine the oil content (EN ISO 659, 2009).

Phospholipids

Part (10 g) of the air-dried fruit was subjected to Folch extraction according to Christie (Christie, 2003). Polar lipids were isolated from the total lipids by column chromatography. Briefly, the sample (100 mg) was applied on a 40 cm x 2 cm glass column packed with Silica gel Unisil 100-200 mesh (Clarkson Chemicals Co., USA) and eluted in sequence with chloroform (for neutral lipids, sterols and sterol esters), and with methanol to isolate phospholipids. The phospholipid classes were isolated by a variety of the two-dimensional thin-layer chromatography on 20 cm x 20 cm glass plates with 0.2 mm Silica gel 60 G layer (Merck) impregnated with aqueous $(NH_4)_2SO_4$ (10 g/kg). In the first direction the plate was developed with chloroform : methanol : ammonia, 65 : 25 : 5 (by volume) and in the second – with chloroform : acetone : methanol : acetic acid : water, 50 : 20 : 10 : 10 : 5 (by volume) (Schneiter, 2006). The individual phospholipids were detected and identified by spraying with specific reagents according to Christie (Christie, 2003): Dragendorff test (detection of choline-containing phospholipids); Ninhydrin spray (for phospholipids with free amino groups), and Shiff's reagent (for inositol containing phospholipids). Additional identification was performed by comparing the respective R_f values with those of authentic commercial standards subjected to Silica gel TLC under identical experimental conditions. The quantification was carried out spectrophotometrically against a standard curve by measuring the phosphorous content at 700 nm after scrapping the respective phospholipid spot and mineralization of the substance with a mixture of perchloric acid and sulphuric acid, 1:1 (by volume). The calibration curve was constructed by using a standard solution of KH_2PO_4. It was linear in the concentration range 1 – 130 μg/ml (as phosphorus). In each series of measurement a standard solution of KH_2PO_4 (10 μl/ml in water) was used to confirm the validity of calibration.

Sterols

The glyceride oil (sample size of 100 mg, precisely measured) was applied on 20 cm x 20 cm glass plates (ca. 1 mm thick Silica gel G layer) and developed with n-hexane : acetone, 100 : 8 (by volume). Free (R_f = 0.4) and esterified sterols (R_f = 0.8) were detected under UV light by spraying the edges of each plate with 2´,7´-dichlorofluorescein, they were then scraped, transferred to small glass columns and eluted with diethyl ether. The solvent was evaporated under a stream of nitrogen and the residue was weighed in a small glass container to a constant weight. Free sterols were then subjected to gas chromatography (GC), without derivatization. Sterols esters were hydrolized with ethanolic KOH, sterols were extracted with n-hexane and purified by TLC under the above conditions prior the GC analysis (ISO 12228, 1999). The sterol composition was determined on HP 5890 gas chromatograph (Hewlett Packard GmbH, Austria) equipped with a 25 m x 0.25 mm DB - 5 capillary column (Agilent Technologies, Santa Clara CA, USA) and flame ionization detector. The temperature gradient varied from 90°C (hold 2 min) up to 290°C at a rate of change 15°C/min and then up to 310°C at a rate of 4°C/min (hold 10 min); the injector temperature was 300°C and the detector temperature was 320°C. Hydrogen was the carrier gas at a flow rate $1.3(3).10^{-8}$ m^3/s; split 100:1. Identification was confirmed by comparison of retention times with those of a standard mixture of sterols.

Tocopherols

Tocopherols were determined directly in the oil by high performance liquid chromatography (HPLC) (ISO 9936, 1997) on a Merck-Hitachi (Merck, Darmstadt, Germany) instrument equipped with 250 mm x 4 mm Nucleosil Si 50-5 column (Merck, Darmstadt, Germany) and fluorescent detector Merck-Hitachi F 1000. The operating conditions were as follows: mobile phase of n-hexane : dioxan, 96 : 4 (by volume), flow rate $1.6(6).10^{-8}$ m^3/s, excitation 295 nm, emission 330 nm. A 20 μkl solution of crude oil (10 g/kg) was injected. Tocopherols were identified by comparing the retention times with those of authentic individual pure tocopherols. The tocopherol content was calculated based on the tocopherol peak areas in the sample vs. tocopherol peak area of standard tocopherol solution.

Fatty acids

The total fatty acid composition as well as the fatty acid composition of phospholipids and sterol esters was determined by GC (ISO 5508, 2000) after transmethylation of the respective sample with 2N methanolic KOH at 50^{o}C according to Christie (Christie, 2003). Fatty acid methyl esters (FAME) were purified by TLC on 20 cm x 20 cm plates covered with a 0.2 mm Silica gel 60 G layer with mobile phase n-hexane : acetone, 100 : 8 (by volume). GC was performed on a gas chromatograph equipped with a 30 m x 0.25 mm x 25 μm (I.D.) capillary EC 30-Wax column (Hewlett Packard GmbH, Austria) and a flame ionization detector. The column temperature was programmed from 130^{o}C (4 min), at 15^{o}C/min to 240^{o}C (5 min); injector and detector temperatures were 250^{o}C. Hydrogen was the carrier gas at a flow rate 1.3(3).10^{-8} m^{3}/s; split was 50:1. Identification was performed by comparison of retention times with those of a standard mixture of FAME subjected to GC under identical experimental conditions.

Extraction of alkaloids from Gleditsia triacanthos leaves

Dried plant material was ground into a blender to a particle size of 2-3 mm. 200 g milled material was mixed with 1 L of 10% ammonia solution. The mixture was stirred for 20 min, then adding 300 ml of dichloroethane (DCE) and stirred at room temperature for 5 hours. The extract was filtered under vacuum. The organic layer was separated from the obtained filtrate. The process of extraction was repeated three times. The combined extracts of DCE were evaporated in vacuo to a volume of 300 ml. The DCE extract was extracted three times (3 x 15 ml) with solution of 10% sulfuric acid. Then the water-soluble fraction was alkalized by 25% NH_3 to pH=8. A precipitate of white crystals of triacanthine is formed. The crystals were filtered under vacuum and washed with cold water.

0.006 g from the obtained crystals we dissolved in 1 ml methanol. Then we injected 1 μl into the GC/MS for analysis. This method has been used successfully for identification and characterization of the alkaloid triacanthine.

Gas chromatography/mass spectrometry (GC/MS) analysis of triacanthine

GC/MS analysis was carried out using Thermo Finnigan GC system combined with Trace GC/Trace DSQ MS (quadrupole) with electron impact ionization (70 eV). Agilent J&W DB-5-MS (5% phenyl methyl siloxane) column (30 m 0.25 mm I.D, film thickness 0.25 μm) was used. The column temperature was programmed to rise from 0^{o}C to 120^{o}C 1 min isothermal, from 120^{o}C to 190^{o}C at a rate 20^{o}C/min, from 190^{o}C to 260^{o}C at a rate 3^{o}C/min from 260^{o}C to 300^{o}C at a rate 25^{o}C/min; 4 min isothermal. The carrier gas was helium adjusted to a linear velocity of 1 ml/min. Injector splitless-250 ^{o}C and injection volume 1μl.

Extraction of Polyphenols from Gleditsia triacanthos leaves

The milled plant material was weighed with 0.0001 g precision, taking 0.2 g samples for Gleditsia samples. Five replications of every sample were prepared. The flavonoids were analyzed in their glycoside form and therefore nonhydrolysed plant extracts were prepared. The powdered Gleditsia samples were extracted with 10 ml 70% (v/v) aqueous methanol. All extracts were sonicated for 40 min and then they were filtrated under vacuum. The volume of all samples was adjusted to 10 ml and passed through a membrane filter 0.45 μm prior to HPLC analysis.

HPLC – PDA Analysis

The instrumentation used for HPLC analysis consisted of quaternary mixer Smartline Manager 5000, pump Smartline 1000 and Photo diod detector (PDA) 2800 detector (Knauer, Germany). Columns was used: a Purospher C18, 25 cm x 4.6 mm I.D, 5 μm particle size (Merck, Germany). Mobile phase flow rate was set by 1.0 ml/min; sample volume was 20 μl.

The method was employed to separate the polyphenols using acetic acid. The method of separation used gradient elution. Solvent A mixture from 95 parts 1% acetic acid and 5 parts methanol. Solvent B mixture 15 parts 1% acetic acide and 85 parts methanol. The following gradient elution program: 0-10 min, 100%-80% A (0-20% B), 10-35 min, 45% A (55% B), 35-55 min, 0% A (100% B).

The polyphenols were detected at 320 nm (maximum absorption for caffeoylquinic acids), 352 nm (maximum absorption for rutin luteolin and quercetin) and at 340 nm (maximum absorption for apigenin).

Statistical Analysis

All the analyses were made in triplicate. Statistical differences between samples were tested using ANOVA. Dates were expressed as mean ± SD. The level of significance was set at $p<0.05$.

3. RESULTS AND DISCUSSION

General characteristics of the oil

General characteristics of the seeds were determined such as: oil content in dry seeds, content of total phospholipids, sterols and tocopherols in the oil and the seeds. The results are shown in Table 1.

Table 1.General composition of *Gleditsia triacanthos* in triglyceride oils and seeds

Compounds	Content
Glyceride oil in the seeds, %	4.6
Phospholipids, %	
• In oil	4.8
• In seeds	0.03
Sterols, %	
• In oil	3.1
• In seeds	0.13
• In unsaponifable compounds	24.7
Tocopherols, mg/kg	
• In oil	2498
• In seeds	115
Unsaponifable compounds, %	
• In oil	11.4
• In seeds	0.5

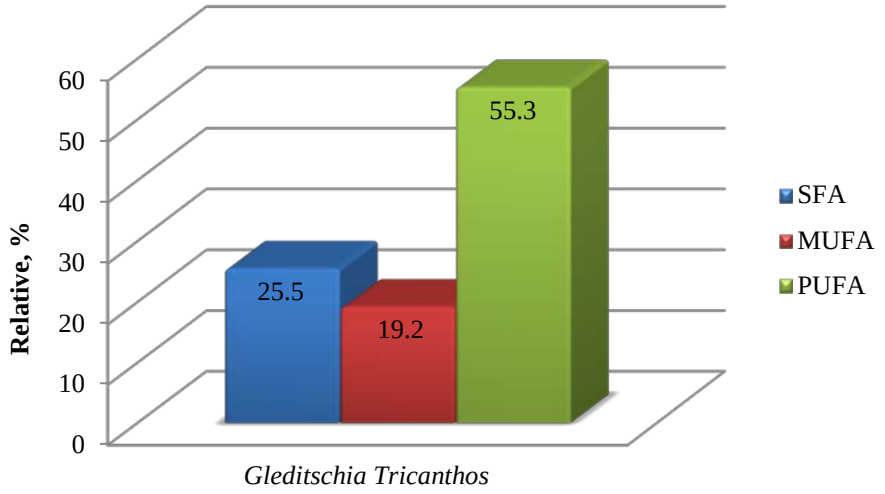

Fig. 1. Ratio of SFA (saturated fatty acids), MUFA (monounsaturated fatty acids) and PUFA (polyunsaturated fatty acids) in seed oil

The content of glyceride oil in the seeds was 4.6%. This value was close to data reported earlier by (Mariod, 2008). Very high percentage of phospholipids, sterols and tocopherols were determined in the glyceride oil. These quantities were several times higher than their contenta in other vegetable oils as sunflower and rape seed oil (Schwartz et al., 2008).

The correlation unsaturated: saturated fatty acids was 74.5: 25.5. Their distribution profiles are presented on Figures 1.

13 types of fatty acids were detected in the oil. Linoleic acid (54.5%) predominate in the oil followed by palmitic (17.1%), oleic (18.6) and stearic (7.5%) acid. This composition was different from data reported by Mariod, 2008, where palmitic and oleic were in approximately equally quantities (34.4% and 38.4% respectively). The main part of the oil were polyunsaturated fatty acids (55.3%). Monounsaturated and saturated fatty acids were 19.2% and 25.5% respectively. This profile defines *Gleditschia triacanthos* glyceride oil as linoleic variety like to cotton oil which was caracterised with similar content of palmitic and linoleic acid (Codex alimentarius, 2005).

The composition of phospholipid fraction was presented in Figure 2.

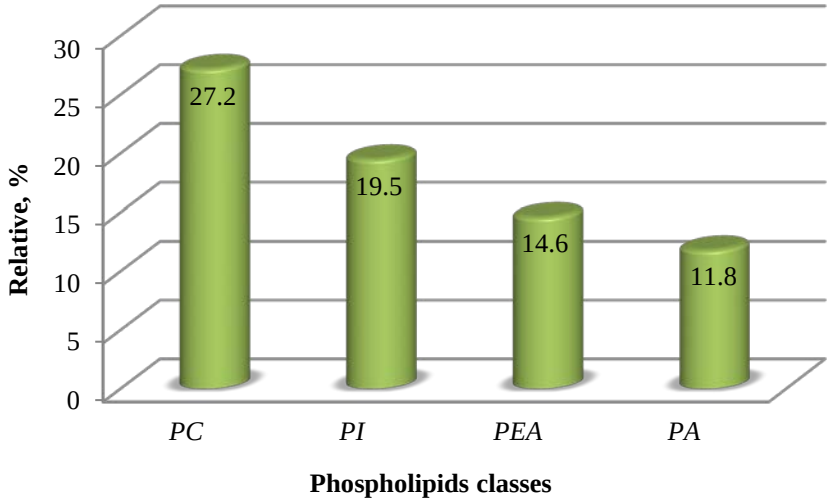

Fig. 2. Phospholipid classes in seed of *Gleditsia triacanthos*

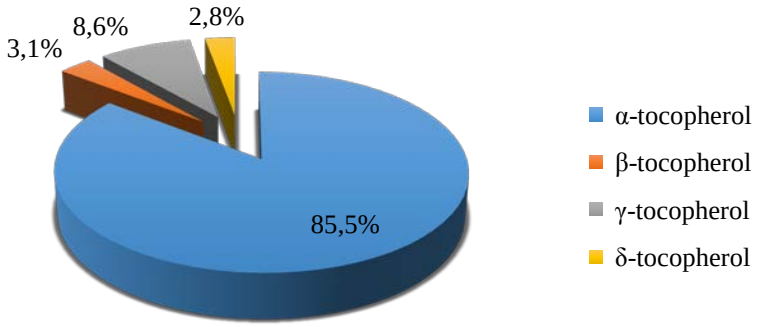

Fig. 3. Tocopherol composition of seed oil from *Gleditsia triacanthos*

Highest content of phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acids was established in the oil. The other constituents were detected in insignificant quantities.

In sterol fraction was determined mainly β-sitosterol (86.3%) at the expense of low content of campesterol and stigmasterol. On the other hand relatively high value of cholesterol was detected. This composition was different from other vegetable oils where the content of campesterol and stigmasterol was several times higher and the content of cholesterol lower than the obtained data about *Gleditschia triacanthos* (Abadi, 2001).

α -Tocopherol predominates in tocopherols fraction (Figure 3) (85.5%), followed by β- γ- and δ- tocopherols but it was not detected tocotrienols derivatives.

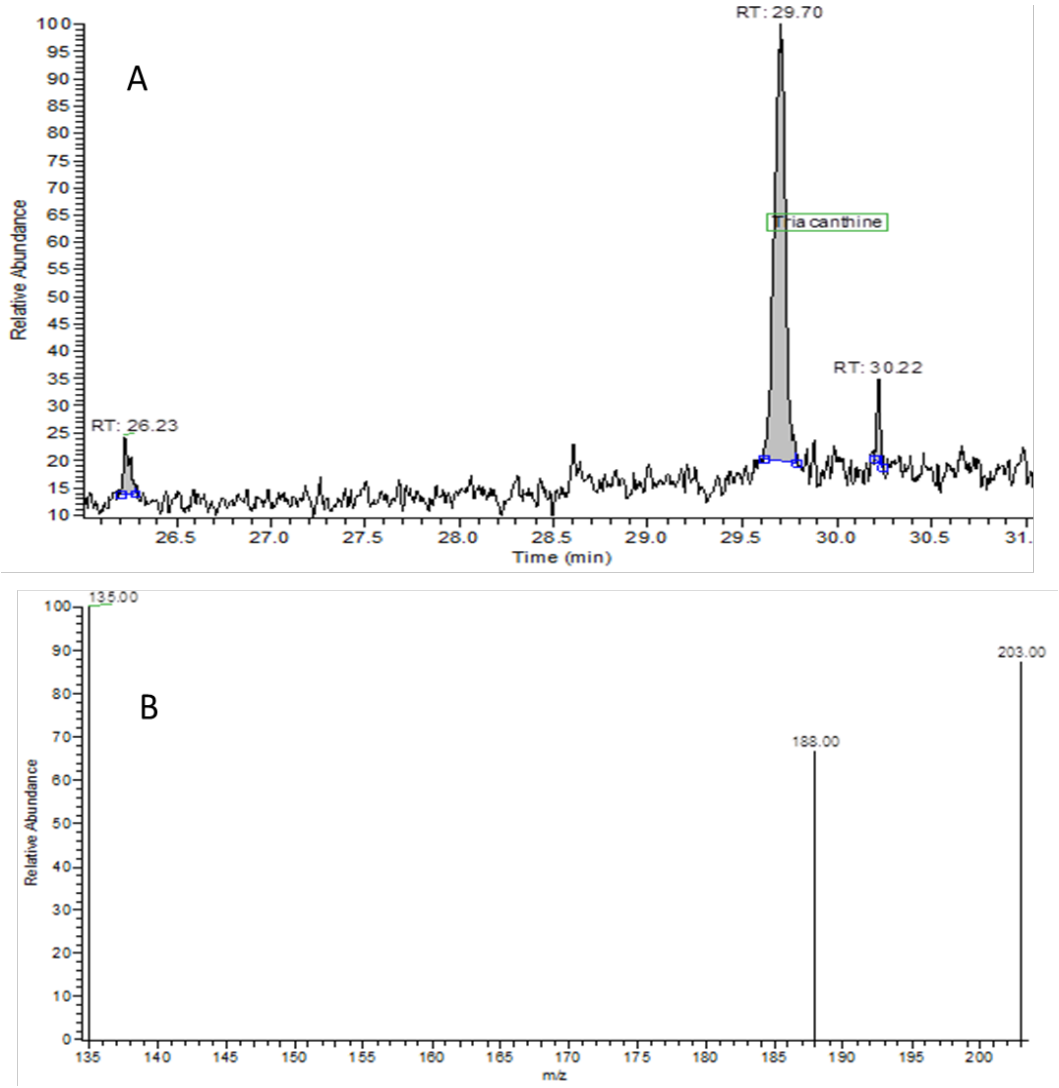

Fig. 4. Chromatographic profil and mass spectrum of alkalod triacanthine. A: GC chromatogram of alkaloidal fraction of *Gleditsia triacanthos*. The retention time of triacanthin is 29.70 min. B: Mass spectrum of triacanthine. The characteristic ions are: m/z 135 (100), m/z 188 (62), m/z 203 (71)

Analysis of triacanthine

The GC/MS analysis of underivatized alkaloid from Gleditsia extracts was performed using an DB-5 MS column. The identification of alkaloid was obtained by comparison of mass spectra with those from literature data (Leonard, 1962). The most abundant alkaloid in the sample is triacanthine, which was identified by

comparison with its characteristic mass fragmentation pattern. The retention time of triacanthine is 29.70 min. and it is a dominant peak in the sample (Fig. 4A).

The mass spectrum showed peaks at 203, 188 and 135 (Fig. 4B). The peak at mass number 230 was assigned to the $C_{10}H_{13}N_5^+$ ion. The peak at mass number 188 was apparently due to the loss of a methyl group producing a $C_9H_{10}N_6^+$ ion. Consequently, triacanthine has to possess at least one methyl group, a fact confirmed in part by a Kuhn-Roth C-methyl determination. The fragmentation represented by the peak in the mass spectrum at 135 ($C_6H_5N_6^+$), which was suggestive of the presence in the original alkaloid of an aromatic ring system containing all five nitrogens, was realized in other reactions (Leonard, 1962).

Analysis of Polyphenols

To give the most chemical information and best separation in the chromatograms, the mobile phase and its flow rate, conditions for elution, column temperature and detection wavelength were investigated in this study. The chromatographic conditions were optimised using samples based on conditions given in literature report (Dagnon et al., 2013). In the performance of gradient optimisation, gradient time, gradient shape and initial composition of the mobile phase were taken into consideration. Considering system back pressure and running time, the flow rate was set at 1.0 ml/min when column temperature was kept at 25 °C. Under the optimal conditions, phenolic acids, rutin, apigenin and other components in the extracts of *Gleditsia triacanthos* sample were well separated. The use of HPLC-PDA method produced a significant increase in peak capacity.

The identity of the chromatographic peaks was confirmed by comparison of the retention times of the samples with those of the standard compounds (Fig. 5). The UV spectral characteristics of the eluting peaks, scanned with diode-array detector (λ=200-400 nm) were compared with those of the authentic standards. Peak assignments for those components where no references were available were based on data for retention time according to the literature and the quantification was done by using the calibration curves of chlorogenic acid, rutin, quercetin, luteolin and apigenin. The content of the components was determined by the external method using a calibration curves established with five dilutions of each standard with correlation coefficients between 0.9994 and 1. The relative standard deviation (RSD) of the methods varied between 3% and 20% by the different components. The limit of detection (LOD) of the polyphenols from 0.5 μg/ml to 0.7 μg/ml. The limit of quantification (LOQ) differed from 1.5 μg/ml to 2.1 μg/ml. Each sample was analyzed several times (min 3), and the mean value was used for calculation. The results are expressed as mean ± SD.

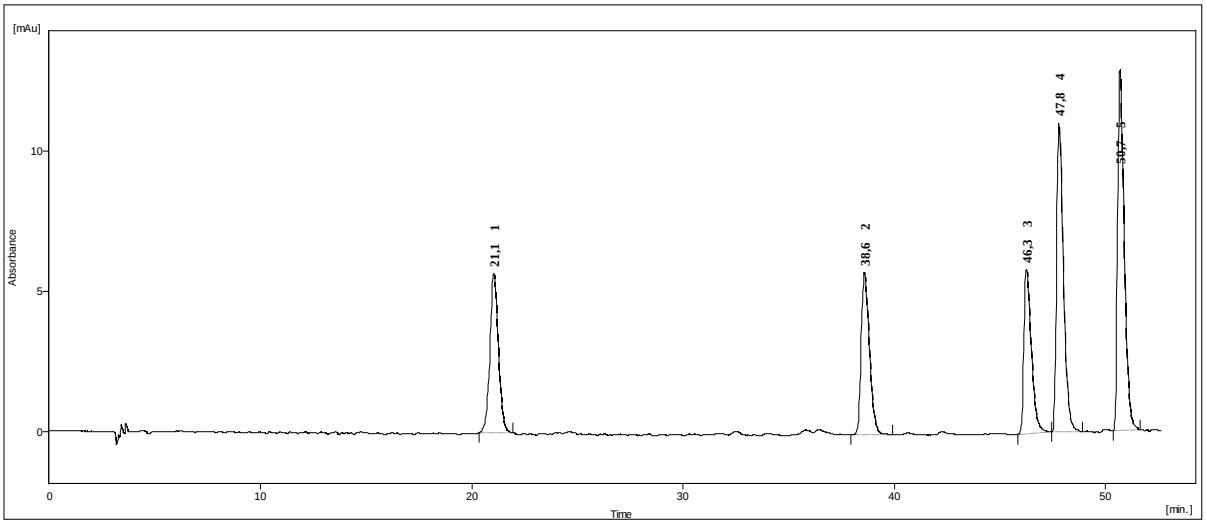

Fig.5. HPLC profile of standart compounds: 1-chlorogenic acide; 2-rutin; 3-luteolin; 4-quercetin; 5-apigenin

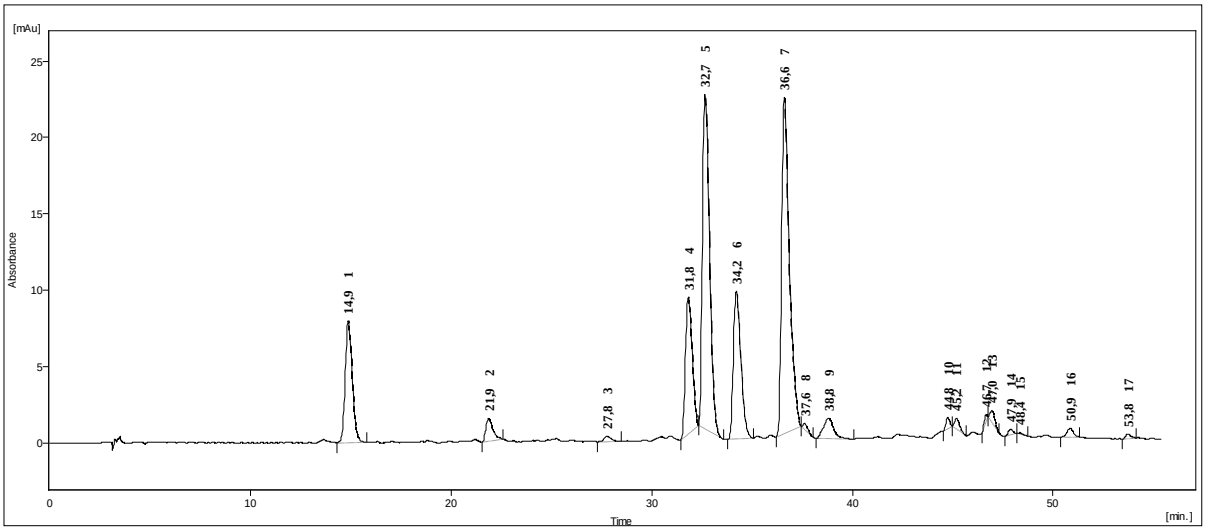

Fig.6. HPLC profile of *Gleditsia triacanthos* extract. Peak: 1-neochlorogenic acid; 2-chlorogenic acid; 4-myricetin-3-O-rutinoside; 5-myricetin-3-O-glucoside; 6-apigenin-glycoside; 7-apigenin-7-O-glucoside; 9-rutin; 10-isorhamnetin-3-O-gentiobioside; 11-isorhamnetin-3-O-glucoside; 12-luteolin; 14-quercetin; 16-apigenin

Table 2. Content of polyphenolic compounds (mg/g) in dry leaves of *Gleditsia triacanthos*

Polyphenols	(mean ± SD), mg/g	RSD, %	LOD, µg/ml	LOQ, µg/ml
NeO	1.58 ± 0.05	3	0.7	2.1
ChA	0.35 ± 0.063	18	0.7	2.1
Rut	0.33 ± 0.066	20	0.7	2.1
Lut	LOD		0.7	2.1
Qrc	LOD		0.5	1.5
Apg	LOD		0.5	1.5

From the analysis we found low concentration of aglycones luteolin, quercetin and apigenin then caffeoylquinic acids (Table 2). The neochlorogenic acid was the major caffeoylquinic acid in the specimen of *Gleditsia triacanthos*. According scientific literature the glycosides predominate in *Gleditsia* species. For the identification of the main components of *Gleditsia triacanthos* we used literature data. Kovalev and Duchenko (Kovalev et al., 2009) contribute to the analysis of *G. tricanthos*. They have been identified the flavonoid glycosides, which are the main components in this plant. Comparing the reference fingerprint and chromatographic profile of the sample showed a high degree of similarity. Through this approach, we established that peaks 4, 5, 6, 7, 10 and 11 are myricetin-3-O-rutinoside; myricetin-3-O-glucoside; apigenin-glycoside; apigenin-7-O-glucoside; isorhamnetin-3-O-gentiobioside; isorhamnetin-3-O-glucoside respectively (Fig. 6). On Fig. 6 the chromatographic profiles of *G. triacanthos* is shown. The separation of the polyphenols of *Gleditsia triacanthos* pointed to the predominant presence of flavonoid glycosides (peaks 4-11).

This study proved that the content of caffeoylquinic acids, flavonoid glycosides and aglycones confirmed by scientific periodicals.

4. CONCLUSION

The seeds of *Gleditsia tricanthos* in comparison with other vegetable contain relatively low quantity of glyceride oil but the oil was very rich in biologically active substances as essentially fatty acids, phospholipids, sterols and tocopherols.

For the identification of pharmacologically active alkaloid in *Gleditsia triacanthos* L the GC–MS method was developed. This method is suitable for assay and quality control of Gleditsia raw materials and products, but could also be valuable tools for the chemotaxonomic evaluation of the species.

In this study, we developed a liquid chromatography diode array detector method for comprehensive identification and characterization of flavonoids in *Gleditsia triacanthos*. The identification and evaluation were performed by comparing the retention time and UV spectra of the samples with standards. This method is suitable for identification and characterization of various flavonoids in Gleditsia plants. The method is very useful for further chromatographic fingerprint of flavonoids obtained from medical plants.

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