Soild-Liquid Extraction Kinetics of Total Phenolic, Total Flavonoid, Rutin and Tannin Contents in 50% Ethanol Extract of *Cotinus coggygria*

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Abstract— The use of a natural product with therapeutical properties has a long history. Ethanolic extract of *Cotinus coggygria* were analyzed for its phytoconstituents show that the dry leaves of *Cotinus coggygria* are rich in total phenols, total flavonoids, rutin and tannins. Solid–liquid extraction was carried out by 50% aqueous ethanol for 120 min, which gave concentration of total polyphenols and the findings of our present results are within good agreement with the other workers.

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Index Terms— Rutin, Tannins, Total flavonoids, Total phenolics, Solid–liquid extraction, 50% aqueous ethanol extract of Bulgarian dry leaves of *Cotinus coggygria*

1 INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. Plants play a significant role in providing primary health care. They serve as therapeutic agents as well as important raw materials for the manufacturing of traditional and modern medicines as well as in food industries. The revival of interest in plant derived drugs is mainly due to the current widespread belief that "green medicine" is safer and more dependable than the costly synthetic drugs mainly of which have adverse side [1, 2]. The medicinal value of these plants lies in some chemical constituents that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, phenolic compounds etc [2, 3].

In recent years there is an increasing interest in finding antioxidant phytochemical because they inhibit the propagation of few diseases like atherosclerosis, diabetes, arthritis, cancer, Alzheimer, ageing, neurogenerative diseases, etc [2, 4]. The interest in the investigation of active components, especially polyphenols, from natural sources (fruits, vegetables, cereals, herbs) has greatly increased in recent years. The reason for that is restricted use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) in foods because of their possible undesirable effects on human health [5, 6]. The free radicals are also produced by oxidation of lipids of foods is responsible for the formation of off-flavors and undesirable chemical compounds which may be harmful to health so antioxidants are used by the food industry to delay the oxidation process.

Antioxidants act by donating H-atom or by donating electrons to radical oxygenated species. Radicals obtained from antioxidants with molecular structure such as phenol, are stable species and will stop the oxidation chain reactions [2, 7]. Many studies had revealed that phenolic content in plants is mainly responsible to their antioxidant activities. Higher the total phenolic content greater is the antioxidant power [2].

Cotinus coggygria is one of two species constituting a minor genus of the family Anacardiaceae, viz., *Cotinus coggygria* Scop. (syn.: *Rhus cotinus* L.) itself and *Cotinus obovatus* Raf., the American smoketree. Its wide distribution extends from southern Europe, the Mediterranean, Moldova and the Caucasus, to central China and the Himalayas [8, 9]. *C. coggygria* is a common medicinal plant (well known as 'smradlika' or 'tetra') in the Bulgarian folk medicine for outer use predominantly [10, 11]. Plants of the family Anacardiaceae have a long history of use by various peoples for medicinal and other purposes. *Rhus glabra* is traditionally used in the treatment of bacterial diseases such as syphilis, gonorrhea, dysentery and gangrene, while *R. coriaria*, besides its common use as a spice consisting of ground dried fruits with salt, is also widely used as a medicinal herb, particularly for wound healing [9, 12]. In folk medicine, *Cotinus coggygria* is routinely used as an antiseptic, anti-inflamatory, antimicrobial and antihaemorragic agent in wound-healing [12, 13], as well as for countering diarrhea, paradontosis, and gastric and duodenal ulcers [12, 14]. However, these by-products are still a good and cheap source of high-quality polyphenolic compounds which can be used in different therapeutic procedures with the purpose of free radical neutralisation in biological systems [6,15, 16].

Extraction is a very important stage in isolation, identification and use of phenolic compounds and there is no single and standard extraction method. Solid–liquid extraction of phenolic compounds with different solvents [6,17,18,19] and in some cases extraction with supercritical fluid [6,20] are the most common by used techniques for isolation of these compounds. Many authors investigated solid–liquid extraction of natural antioxidants and their properties from grape seed [5, 6, 16]

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and from other plant materials [6, 21, 22, 23] as well as methods for their identification [6, 24, 25]. However, literature data about optimisation [6, 26, 27], modelling and simulation of solid–liquid extraction process are scarce. Therefore, there is a need for mathematical modelling, as a useful engineering tool, which considerably facilitates optimisation, simulation, design and control of processes and contributes to utilization of energy, time and solvent [6].

2 Materials and Methods Used

2.1 Plant Materials

The leaves from *Cotinus coggygria* were harvested from different regions of Bulgaria. All sample data are stated in the sampling protocol. The dried leaves were kept in a dry place until further use.

2.2 Sample Preperation

A dry simple of 0.5 g was weighted and phenolic and flavonoid compounds were extracted with 50 ml 50% aqueous ethanol on an ultrasonic bath for 20 min. An aliquot (2 mL) of the extracts was uitracentrifugated for 5 min at 14 000 rpm. The extract prepared in this way was used for further spectrophotometric determination of total polyphenols.

2.3 Sample Preperation

Determination of total phenolics assay

The total phenolic content of *Cotinus coggygria* were determined by using the Folin-Ciocalteu assay. An aliquot (1 mL) of extracts or standard solution of gallic acid (10, 20, 40, 60, 80, 100 and 120 mg/L) was added to 25 mL volumetric flask, containing 9 mL of distilled deionised water (dd H_2O). A reagent blank using dd H_2O was prepared. One milliliter of Folin-Ciocalteu,s phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na_2CO_3 solution was added to the mixture. To the solution the dd H_2O was added up to volume of 25 mL and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates [28].

Determination of total flavonoids assay

The total flavonoid contents were measured by aluminum chloride colorimetric assay. An aliquot (1 mL) of extracts or standard solution of catechin (10, 20, 40, 60, 80, 100 and 120 mg/L) was added to 10 mL volumetric flask, containing 4 mL of distilled deionised water (dd H_2O). To the flask was added 0.3 mL 5% $NaNO_2$. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. At 6^{th} min, 2 mL 1 M NaOH was added and the total volume was made up to 10 mL with dd H_2O. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates [28].

Rutin assay

The analyses of rutin content in *Cotinus coggygria* were performed according to The International Pharmacopoeia and AOAC method, after modified methods with 50% aqueous ethanol. Pipet 2 ml aliquots solution into 50 ml volumetric flask was added to 2 ml deionized water (dd H2O) and 5 ml ammonium molybdat. The solution was added volume (50 ml) with dd H2O and mixed. Was prepared standard solution of rutin (0.0200 g dissolved into 2 ml ethanol) was added volume (50 ml) with 50% aqueous ethanol. An aliquot (1 ml) of standard solution into 50 ml volumetric flask and dilute to volume with distilled deionized water (dd H_2O). A reagent blank using dd H_2O was prepared. The absorbance against prepared reagent blank was determined at 360 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates [29].

Calculations are based on averaging results from analyses of duplicate samples.

Calculate content (%) of rutin (R) in sample as follows:

$$R(\%) = \frac{A_{sample} \times C \times 50 \times 100}{A_{stand} \times W \times 2}$$

where:

A_{sample} - Absorbance of sample was determined at 360 nm,

A_{stand} - Absorbance of standard solution was determined at 360 nm,

C – Concentration of standard solution of rutin (g/ml),

W – weight (g) of sample for analyses,

2 – Volume (ml) of sample for analyses, 100 – Percent, %.

Tannins assay

The analyses of tannins content in *Cotinus coggygria* were performed according to The International Pharmacopoeia and AOAC method, after modified methods. Measured 25 ml of this infusion into 1 L conical flask and add 25 ml indigo solution and 750 ml distilled deionized water (dd H_2O).Titred with 0.1 N water solution of $KMnO_4$ until blue solution changes to

green, then add a few drops at time until solution becomes golden yellow. Was prepared standard solution of Indigo carmine (dissolve 6 g indigo carmine in 500 ml distilled deionized water (dd H_2O) by heating, cool add 50 ml 96% - 98% H_2SO_4, diluted to 1 L and filter). For the blank similarly titred mixture of 25 ml indigo carmine solution and 750 ml dd H_2O. All samples were analyzed in duplicates [30].

Calculations

Calculations are based on averaging results from analyses of duplicate samples.

Calculate content (%) of tannins (T) in sample as follows:

$$T(\%) = \frac{(V - V_0) \times 0{,}004157 \times 250 \times 100}{g \times 26}$$

where:

V – Volume of 0.1 N water solution of $KMnO_4$ for titration of sample, ml;

V_0 – Volume of 0.1 N water solution of $KMnO_4$ for titration of blank sample, ml;

0.004157 – Tannins equivalent in 1 ml of 0.1 N water solution of $KMnO_4$;

g – Mass of the sample for analyses, g;
250 - Volume of volumetric flask, ml; 100 – Percent, %.

3 Statistical Analyses

All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out in duplicate or triplicate. The statistical parameters are calculated in terms of the reproducibility of the experimental data using a statistical package universal ANOVA.

4 Results and Discussion

Dry matter content in all experimental runs was determined and results were expressed on dry basis, which generally provides more accurate and reliable data comparison. The 50% ethanol extract of *Cotinus coggygria* showed the Table 1 and Table 2 of total phenols, total flavonoids, rutin and tannins in qualitative chemical analysis. Solid–liquid extraction was carried out by 50% aqueous ethanol for 120 min, which gave concentration of total polyphenols. The content for total phenolics and total flavonoids of *Cotinus coggygria* varied between 16.77 mg GAE/ml for 10 minutes and 24.11 mg GAE/ml for 120 minutes and 5.66 mg CE/ml for 10 minutes and 8.16 mg CE/ml for 120 minutes. This is shown in the (Table 1) using the gallic acid and catechin as standards. These results indicate that the higher antioxidant activity of the 50% ethanol extract of the *Cotinus coggygria* in 90 min and stopped in 120 min. than the 50% ethanol extract of *Cotinus coggygria* in 10 min may be in correlation with the phenolic and flavonoid contents of the extracts of the leaves.

TABLE 1
THE KINETIC VARITIES OF 50% ETHANOL EXTRACT OF IN THE TOTAL PHENOLS AND TOTAL FLAVONOIDS

min	Total phenols, mg/ml dw	Total flavonoids, mg/ml dw
10	16.77±0.12 RSD 8.9% (n=3)	5.66±0.02 RSD 11.9% (n=3)
15	16.95±0.12 RSD 8.8% (n=3)	5.75±0.02 RSD 11.7% (n=3)
20	19.53±0.12 RSD 8.7% (n=3)	6.47±0.03 RSD 11.4% (n=3)
30	20.22±0.15 RSD 9.3% (n=3)	6.76±0.10 RSD 11.9% (n=3)
60	22.83±0.16 RSD 9.9% (n=3)	7.65±0.11 RSD 11.7% (n=3)
90	24.11±0.21 RSD 10.7% (n=3)	8.16±0.15 RSD 11.8% (n=3)
120	24.11± 0.21 RSD 10.7% (n=3)	8.16±0.16 RSD 11.9% (n=3)

The presence of rutin and tannins in 50% ethanol extract of *Cotinus coggygria* is significant. The content for rutin and tannins varied between 1.39 percent for 10 minutes and 2.26 percent for 120 minutes of rutin and tannins it was found that it was much higher than the content in 50% ethanol extract of *Cotinus coggygria* in 120 minutes between 10.45% and 7.33% in 10 minutes, respectively). The results were shown in the (Table 2), where the data was received using rutin as standard and Potassium permanganate as titrant. It is important to notice that the comparison of the results for rutin and tannin contents in 50% ethanol extract of *Cotinus coggygria* will not be correct because of the different methods of analysis. The results and extraction curves indicated the exponential growth of extraction rate in the time for all examined particle classes.

TABLE 2
THE KINETIC VARITIES OF 50% ETHANOL EXTRACT OF COTINUS COGGYGRIA IN RUTIN AND TANNINS

min	Rutin, %	Tannins, %
10	1.39±0.002 RSD 7.8; n=3	7.33±0.004 RSD 7.9; n=3
15	1.57±0.003 RSD 8.1; n=3	7.74±0.003 RSD 8.5; n=3
20	1.82±0.002 RSD 8.3; n=3	8.58±0.004 RSD 8.6; n=3
30	2.08±0.003 RSD 7.6; n=3	9.21±0.005 RSD 8.1; n=3
60	2.19±0.003 RSD 7.8; n=3	9.82±0.004 RSD 7.7; n=3
90	2.26±0.003 RSD 7.7; n=3	10.45±0.003 RSD 8.9; n=3
120	2.26±0.003 RSD 7.2; n=3	10.45±0.003 RSD 7.5; n=3

The kinetic study was performed by continuously measuring the absorbance of the extract using an UV–VIS spectrophotometer. The continuous measurement is faster and more accurate for kinetic studies of extraction compared to conventional discontinuous methods. In conventional methods, sampling is done manually at given intervals of time, which is not precise, as there is always a time gap between sampling and analysis, which contributes to errors during kinetic measurements.

In all experiments, the extraction yield was significantly time dependant and the profile clearly shows that the yield of *Cotinus coggygria* rises rapidly with time at first, and then less and less quickly as the progress of extraction continues. This behaviour can be attributed to the fact that during the initial stage of extraction, when the solvent penetrates into the solid, an extremely high concentration gradient is developed, resulting in high rates of mass transfer into the liquid phase. As the extraction time increases, the mass transfer of solutes from the solid phase to the fluid becomes more difficult, due to the decrease in concentration driving force between the solid and liquid phases. In addition, as the extraction time proceeds, the concentration of analytes in the solid phase decreases and both the solubility of the mixture and the extraction rate decrease simultaneously [31-33]. In all experiments, a higher extraction yield was observed especially from 10 to 90 min, with a lower yield seen from 90 to 120 min.

The WHO survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, for their primary healthcare [34, 35]. Plants are not only important to the millions of people to whom traditional medicine serves as the only opportunity for health care and to those who use plants for various purposes in their daily lives, but also as a source of new pharmaceuticals [36, 37]. So far the extracts of many plant species have been examined for

a number of biological activities, and their antimicrobial, anti-inflammatory, antioxidant, antimutagenic and cancer preventive effect have been partially described [37-41]. The use of a natural product with therapeutical properties has a long history. Plants are invaluable sources of pharmaceutical products [37, 42]. Many plant extracts have
been used as a source of medicinal agents to cure urinary tract infections, cervicitis vaginitis, gastrointestinal disorders, respiratory diseases, cutaneous affections, helminitic
infections and inflammatory process [37, 43, 44].
These medicinal plants are rich sources for naturally occurring antioxidants especially phenolic and flavonoids contents. These agents have ability to scavenge free radicals, super oxide and hydroxyl radicals, etc thus they enhance immunity and antioxidant defense of the body [45, 46]. Polyphenolic compounds like flavonoids, tannins, and phenolic acids, commonly found in such plants which contain various biological effects including antioxidant acivity [47-49]. Mainly, phenolic compounds are very important for the free-radical scavenging and antioxidant activities of plants because these compounds react as hydrogen donors and thus neutralize the free-radicals [48-51]. Researchers have studied polyphenolic constituents having a potential to medicinal or nutraceutical properties including antioxidant activities [49, 52]. Therefore, the study of the importance and role of nonnutrient compounds, particularly phenolic acids, flavonoids and high molecular tannins as natural antioxidants has greatly increased [49, 53, 54]. There was a linear relationship between the antioxidant activities and phenolic compounds [49, 55, 56] Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being.
Phytochemical investigation of the ethanol extract of plant *C. coggygria* led to the isolation of several phenolic compounds [37, 57, 58]. Polyphenolic compounds are known to have antioxidant activity and it is likely that the activity of the extracts is due to these compounds. It is
suggested that polyphenolic compounds have shown anticarcinogenic effects and potential to prevent cardiovascular and cerebrovascular diseases [37, 59].
Ethanolic extract of *Cotinus coggygria* were analyzed for its phytoconstituents show that the dry leaves of *Cotinus coggygria* are rich in total phenols, total flavonoids, rutin and tannins to some extent (Table 1, Table 2). It is well that plant flavonoids and phenols in general, are the highly effective free radical scavenging and antioxidants. In this present study the phytochemical screening and quantitative estimation of the percentage of chemical constituents of the plants studied showed that the dry leaves of *Cotinus coggygria* were rich in rutin and tannins.
Cotinus coggygria was the most active species among the plants selected for the screening. This plant is largely used in the Balkan and Anatolian regions to cure wounds and reduce inflammations, as well as for the treatment of gastrointestinal and respiratory disorders [60, 61]. In Asiatic countries, *C. coggygria* is also known as a bactericide and frequently administered against hepatitis and even anemia [61, 62]. A relative of this species, *Rhus coriaria*, which grows
in the Mediterranean region, has demonstrated inhibitory properties towards *Streptococcus mutans* and *S. sanguinis*, common components of dental plaque [61, 63]. These authors attributed this effect to the presence of large amounts of tannins in the plant. Tannins can then generate smaller phenolics compounds (pyrogallol, catechol, and ellagic acid) with known bactericidal actions. Similarly, *C. coggygria* is very rich in phenolic compounds [61, 64] and displays a significant antimicrobial activity.
Tannins are polyphenolic substances with various molecular weights and a variable complexity [30, 49, 65]. Tannins exhibit many biologically significant functions, such as protection against oxidative stress, and degenerative diseases [30,49]. Rutin is the glycoside between the flavonol quercetin and the disaccharide rutinose [29, 49]. Rutin is one of the bioactive flavonoid compounds, which are present in substantial amounts in plants.
Phenolic compounds have multiple biological effects, such as anti-atherogenic, antioxidant, anti-inflammatory, cardioprotective, antimicrobial, anticarcinogenic and neuroprotective [66-68]. The contents of total phenolics, tannins and flavonoids of the *C. coggygria* extract and its derived fractions are given in Table 1 and 2. Plant secondary metabolites such as terpenoids, phenolic compounds, alkaloids and lectins exert an antimicrobial effect [68, 69].
Many efforts have been made to discover new antimicrobial compounds from various sources such as animals, microorganisms and plants. Plants possess antimicrobial natural products to protect themselves [37, 69, 70]. Antimicrobial activities of various herbs and spices in plant leaves, flowers, stems, roots or fruits have been reported [37,71- 74].
Microbial antibiotic resistance is increasing worldwide and can be demonstrated against all classes of antibiotics. Moreover, multi-drug-resistant strains have emerged in many species of pathogenic microorganisms [68, 75]. Consequently, there is a growing interest in new sources of antimicrobial agents. The phenolic compounds are not only responsible for the antimicrobial activity of *C. coggygria*. This is in agreement with the previous demonstration of the high antimicrobial effect of the essential oil from C. coggygria leaves on Gram-positive bacteria and fungi [8, 68].
The antioxidant activity of phenolic compounds is based on their ability to donate hydrogen atoms or electrons, chelate metal cations and scavenge free radicals [68, 76, 77]. The process of LP has an important role in oxidative stress development. The peroxidation of unsaturated lipids of the cell membrane leads to cell membrane damage, loss of membrane integrity and consequently to cell and tissue damage [68, 78].
ROS damage cell membranes and DNA, causing potentially cancerous mutations. They are also implicated in inflammatory processes [68, 79]. Phenolic compounds have an ability to inhibit the production of ROS or to neutralise radicals that are involved in the inflammatory process as signalling molecules [68, 80].

5. Conclusion

In conclusion, the results of this research showed that total phenolic, total flavonoid, rutin and tannin contants are important components in 50% ethanol extract of Bulgarian dry

leaves of *Cotinus coggygria* this plant, and some of the pharmacological effects. Results of the present work clearly shows that the yield of *Cotinus coggygria* rises rapidly with time at first, and then less and less quickly as the progress of extraction continues.The Bulgarian dry leaves of *Cotinus coggygria* are animportant component, providing some protective/preventative health effect.

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