FULL PAPER

Extraction and determination of tannic acid in rosemary, anise, and cinnamon by reversal phase RP-HPLC

Halla Talib Salih* |Ameera Hassan Hamed

Department of Chemistry, College of Science for Woman, University of Baghdad, Baghdad, Iraq

A reversed-phase high-performance liquid chromatography method was used for quantitative tannic acid in (rosemary, anise and cinnamon) with isocratic mode. the column which has been used is C18 (250 mm×4.6 mm i.d.;5 µm) at room temperature, with a mobile phase composed of methanol /water which the best ratio for separation tannic acid was (90:10 v: v%) at (pH 6) and the optimum flow rate was 1.5 mL/min, the run time (5 min) at a detection wavelength 277 nm at room temperature. The linearity of calibration curve was (0.1-60 µg mL^{-1}). The value detection of limit and quantification were calculated to be 0.0037 and 0.0122 µg mL^{-1} respectively. The precision and accuracy were (98.18-100.095%). The recoveries provided by the method were ranged between (-2.57-0.09).

*Corresponding Authors:

Halla Talib Salih and Ameera Hassan Hamed

Email: hala.Taleb1205@csw.uobaghdad.edu.iq

Tel.: +9647812789743

Introduction

Medicinal herbs are really essential to health due to the availability of phytocompounds. Alkaloids, glycosides, tannins, flavonoids, and phenolic compounds have become the most useful of these components[1]. Phenolic chemicals are fundamental for plants' hydroxyl radicals recycling and antioxidant effects; they have a wide range of biological activities, which are mostly ascribed to the antioxidant properties in reducing oxidative stress, inhibiting damage, and ion exchange transitional metals. Hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, and singlet oxygen are all reactive oxygen species (ROS) [2]. Tannic acid (TA) is a natural source for phenolic molecule found in a variety of fruits and vegetables. Because of the amount of TA in tea, fruits, and beer, it can have a significant impact on their flavor; it is a crucial characteristic to consider when reviewing and ensuring the quality of these goods. In the pharmaceutical sector, tannic acid is utilized as an ingredient in products that are used to treat burns and diarrhea [3]. Tannic acid is a food product that is always used. Based on the type of food to which it is put, the safe amount ranges between 10 to 400 µg [4]. Tannic reduced the growth of several fungi, yeasts, bacteria, and viruses [5]. The Antioxidant effect for tannic acid is due to its relatively hydrophobic "center" and hydrophilic "crust". Tannic acid and other polyphenol compounds can be found in a variety of beverages and foods. The foods rich in polyphenol, and plants have inhibitory and protective effects on a number of diseases such as cardiovascular disorders, which could be connected least in part to polyphenols antioxidant activity. Tannic acid has been shown to reduce skin, lung, and stomach cancers caused by polycyclic aromatic hydrocarbon carcinogenic. Tannic acid and

some other polyphenols have been shown to have anticancer, antioxidant, and anti-carcinogenic properties. The tannic acid as an antioxidant mechanism, for example, behaves as individual productivity in the condition of copper ions, causing DNA damage, or with an antioxidant, reducing hydroxyl radical generation [4]. It is frequently used as in the healthcare, as a purification for wine and beer in the beverage industry and can be used in leather tanning, and ink compounding [6].

Tannic acid, a type of polyphenol, has a structure with several phenolic hydroxyl groups, giving it great physical and chemical properties as well as amazing pharmacological and biological activity [1]. The chemical formula for tannic acid is $C_{76}H_{52}O_{46}$, (M.wt = 1701) [7]. Figure 1 shows structure of the tannic acid. Tannic acid is a water soluble polyphenol found in a wide range of plants [5].
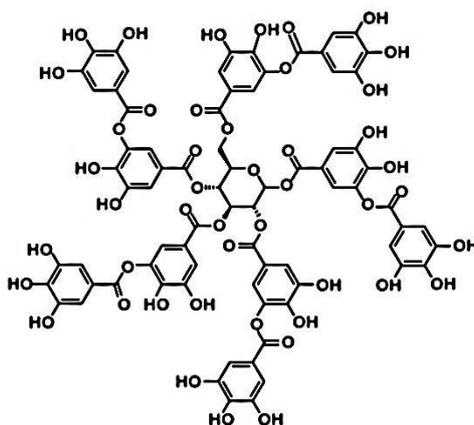

FIGURE 1 Structure of the tannic acid

There are several methods for determination of Tannic acid and they differ according to the samples taken and according to the equipment used for the determination, including high-performance liquid chromatography [8-10], HPLC-mass spectroscopy [11], gas chromatography (GC) [12], Gas-Mass spectroscopy [13,14], flow injection analysis [15-17], and pencil graphite electrode[3,18]. The purpose of this study is to extract and determine the method of estimating tannic acid in some herbal plants for instance Iraqi rosemary, cinnamon, and anise through HPLC and UV detection.

Experimental

Apparatus

A (Shimadzu, UV spectrophotometer U.V-1800, Japan), A (Shimadzue, LC-2010 A, Japan) HPLC instrument with UV-Vis detector, Digital balance, PH meter, (WTW, Ph 720, Germany), Hot plate with Magnetic Stirrer, were purchased.

Reagent and solution

Tannic acid (98%) was provided from (BDH), water deionized. acetic acid (99.5%) from (BDH), potassium hydroxide (≥86%) from (Sigma-Aldrich). All solvents (Methanol and water) for HPLC were used from (CHEM-LAB).

Preparation of stock solution

Tannic acid stock solution 1000 µg mL^{-1} was prepared by dissolving 0.1 gram in methanol and filled to the mark in a 100 mL volumetric flask and diluted. A series of solution ranged (0.1-60 µg mL^{-1}) were prepared to study calibration curve.

Preparation 1% of acetic acid

Acetic acid was prepared by diluting 1 mL of concentrated CH_3COOH in water, then

completed to 100 mL of volumetric flask and diluted.

Preparation 0.5 M of KOH

Prepared 0.5 M of potassium hydroxide was by dissolving 1.4 grams from KOH in water, then completed to 50 mL volumetric flask of water.

Plant material and extraction

Anise was purchased from the local market and has been ground in a mill, 25 grams sample was mixed with 500 mL boiling water by magnetic stirrer for 15 min. Then, the extract was filtered by Whatman No.1 paper. The filtered sample was frozen and the extract was placed in a plastic bottle and then stored at -20 °C until used [19].

Dried cinnamon sticks were purchased from the local market and grounded by mill, 30 grams, sample was mixed with 500 mL boiling water by magnetic stirrer for 30 min. Aqueous extract was double-filtered using Whatman paper No.1. After that, aqueous extract solution transferred to a bottle and freeze until use at (-20 °C) [20,21].

Rosemary, Iraqi rosemary, was purchased from the plantation and its leaves were dried, outside at the ambient temperature (25–30 °C), then inside at a temperature of not more than 40±2 °C. The dried leaves were mechanically cut off and crushed into powder. The extracts were made in a standard manner by boiling 8 grams of dried leaves in 100 mL of distilled water for 5 minutes and then letting them infuse for 10 minutes. The extracts were then chilled and filtered before being used [22].

Results and discussion

Selecting wave length of tannic acid

Prepared stock solution 10 µg mL^{-1} of tannic acid was prepared by dissolving in methanol and scanned from 190-400 nm to choose λ_{max} for tannic acid. 277 nm was detected the best absorbing for tannic acid at concentration of 50 µg mL^{-1}. As shown in Figure 2.
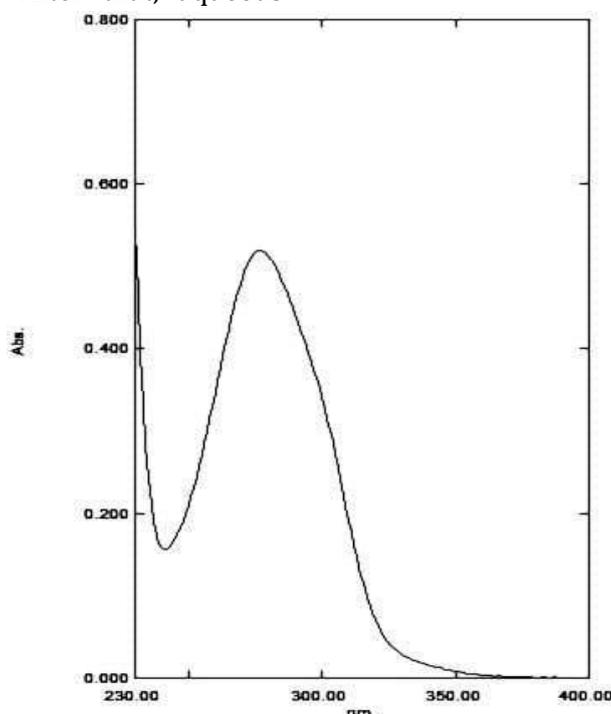

FIGURE 2 Show absorption UV spectra for tannic acid in methanol solvent

Optimization of the experimental conditions for a separation of tannic acid

Effect ratio of mobile phase on elution for tannic acid

Mobile phase composition was methanol: water in different ratios and pump mode was isocratic flow at 1 mL/min at room temperature, the wavelength was detected at 277 nm. Although the ratios of the mobile phase gave a short retention time, they showed a noticeable tailing and irregular and broad peaks that contain a shoulder. The ratio was chosen (90: 10 MET: W v: v%) because of the highest area and height, and lower tailing. Table 1 shows the best ratio.

TABLE 1 Selecting the ratio of mobile phase

MP	Ratio %	(t_R)	Area	Height	k̀	TF 10%
MET-W	90-10	4.686	4851893	97146	24.349	1.570
	80-20	4.536	2598429	56495	0.191	1.888
	70-30	4.490	2170710	37101	0	2.095
	60-40	3.906	1662601	20143	14.552	3.360
	50-50	4.007	945687	19031	15.494	2.070

Effect of pH on retention time elution of tannic acid

To observe the effect of pH change on retention time for separation and determination tannic acid, (TA) was carried out using isocratic elution with mobile phase mobile phase (methanol/water 90:10 v/v %) with 1.0 mL/min flow rate at various pH ranged from (3-7) by using drops from 1% acetic acid. The study shows effect of changing pH on the resolution and response. The best pH 6 has a good peak shape and the highest peak area. The results are shown in Table 2.

TABLE 2 The variation of retention time of tannic acid at different PH

pH	(t_R)	Area	Height	k̀	TF 10%
3	4.932	1749317	41191	75.624	0
3.5	4.712	2325041	60630	0.140	0
4	4.731	2439449	63632	20.945	0
4.5	4.717	3655784	107328	7.080	1.280
5	4.694	4036469	116603	21.777	1.276
5.5	4.669	4036479	112944	29.142	1.331
6	4.683	4139484	112790	24.064	1.366
6.5	4.600	2618359	43131	26.241	0
7	4.590	2530206	47630	0.508	0

The effect of flow rate on elution of tannic acid

Under the ideal conditions discussed earlier, A 20 µl of 50 µg mL⁻¹ tannic acid solution was injected into an HPLC device at various flow rates ranging (0.25- 1.75) mL/min. 1.5 mL/min was chosen because it gave good response, short retention time and the highest peak height and acceptable capacity. The results are shown in Table 3.

TABLE 3 The effect of flow rate on retention time of tannic acid

Flow rate	(t_R)	Area	Height	$\grave{k}$	TF 10%
0.25	9.291	3862674	60022	38.395	1.412
0.5	4.665	2574998	74767	0.314	1.543
0.75	3.116	1900550	82842	12.190	1.577
1	2.356	1598312	84822	13.952	1.619
1.25	1.828	1704801	107160	0.275	1.854
1.5	1.541	1498172	110638	0.241	2.139
1.75	1.369	1157239	102081	0.269	1.411

The Effect of volume injection on separation of tannic acid

Under the earlier optimizations selection of tannic acid, 50 µg mL^{-1} was injected into the HPLC system in various volume ranging from (5 to 20 µL). It was found from the maximum response for injected 20 µL because of the high area and height as shown in Table 4.

TABLE 4 The effect of volume injection on separation of tannic acid

Volume injection (µL)	(t_R)	Area	Height	$\grave{k}$	TF 10%
5	1.671	212190	16711	0.491	1.483
10	1.647	454502	37017	0.475	1.417
15	1.629	695438	57085	0.479	1.546
20	1.624	1054941	84284	0.477	1.816

The best chromatographic conditions

The previous conditions were studied to determine the best parameters, which are the mobile phase (methanol/ water 90:10 v: v%), at PH 6, the flow rate was 1.5 mL/min and the injection volume was 20 µL, detected at 277 nm wavelength at room temperature. Figure 3 shows chromatogram for tannic acid at optimum condition.
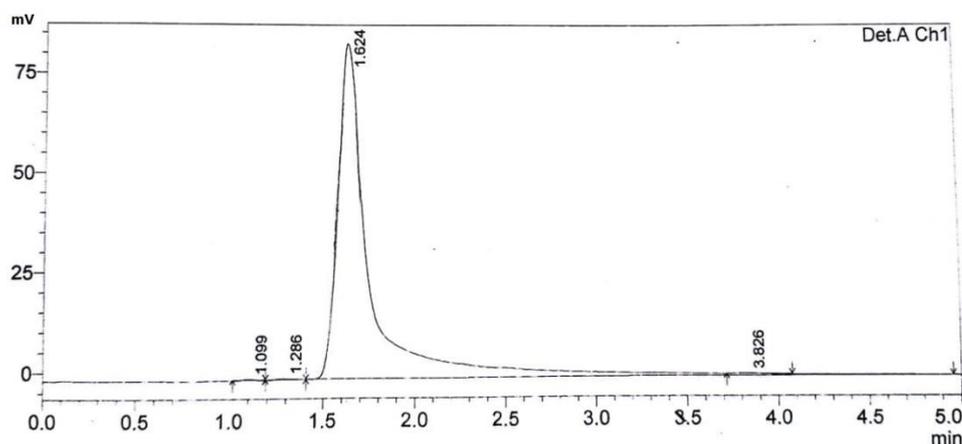
FIGURE 3 Chromatogram for separation of tannic acid at optimum condition

Calibration curve

Separation of calibration plots for tannic acid were constructed by plotting the peak area and peak height against respective concentration by various concentrations of tannic acid (0.1-100 µg mL^{-1}) which were prepared from the standard solution and injected n=3 in the HPLC device under previously optimized conditions. The linearity was ranged from (o.1-60 µg mL^{-1}) as shown in Figure 4. The regression equation, correlation coefficient, limits of detection (LOD) and limits of quantification (LOQ) are given in Table 5.
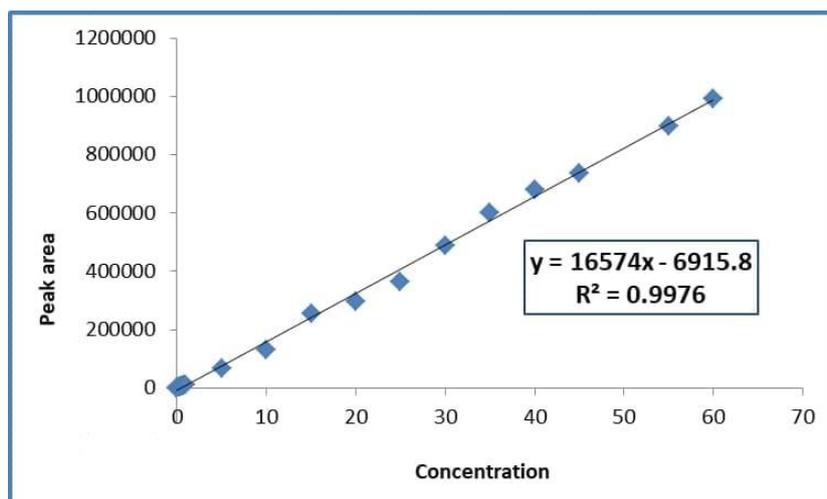
FIGURE 4 Calibration curve for tannic acid standard

TABLE 5 linear regressions data analysis of calibration graph

Parameter	Value
λ_{max}	277
Regression equation	y=16574x - 6915.8
Linearity range (µg mL^{-1})	0.1- 60.0
Slope	16574
Intercept	6915.8
Correlation coefficient (r)	0.9989
Determination Coefficient (R^2)	0.9976
Confidence limit for the slop at 95%	16574±231
Confidence limit for the intercept at 95%	6915.8±6058
Limit of detection (LOD)	0.0037
Limit of quantification (LOQ)	0.0122
Relative standard deviation (RSD%)	6.118

Precision and accuracy

Under the recommended procedure, the accuracy and precision of obtained results for study of tannic acid were evaluated. Three replicated analyses were carried out at three concentrations (10, 20, 40) µg mL^{-1} injected into an HPLC device. The data in Table 6 shows the results which indicate a good accuracy and precision of the proposed method at the studied concentration levels.

TABLE 6 Precision and accuracy of determination of method

Amount. taken (µg mL^{-1})	Amount. found * (µg mL^{-1})	Recovery% *	E$_{rel.}$% *	RSD% *
10	9.818	98.18	-1.82	0.613
20	19.487	97.435	-2.57	12.374
40	40.838	100.095	0.09	2.556

*Average of 3 determinations

Application of method

The proposed method was successfully applied for the determination of rosemary, cinnamon and anise after extracted as mentioned previously; 1 mL of the extract was taken and diluted to 46 mL of distilled water for anise and rosemary [19]. As for cinnamon, 1 mL was diluted to 23 mL of distilled water [21,23-25]. Then, the diluted extract was purified with a 0.45 filter, and was injected into a device under the same conditions as those approved as shown in Table 7.

TABLE 7 Application on anise, cinnamon and rosemary

Sample	(t$_R$)	Area	Conc. of tannic acid (µg mL^{-1})	RSD%*
Cinnamon	1.657	173858	10.073	1.604
Anise	1.623	242301	14.202	1.061
Rosemary	1.666	611193	36.459	0.741

* Average of 3 determinations

Tannic acid extraction from herbs was added to four different concentrations (5, 10, 20, and 30 µg mL^{-1}). Tannic acid analytical values are extract from spiked extracted herbs. The procedure was accurate, with recovery results ranging from (90.39-100.22) and RSD percent ranging from (0.269-2.577) as shown in Table 8.

TABLE 8 Application with spiked sample

sample	Amount added of tannic acid µg mL^{-1}*	Amount spiked of tannic acid µg mL^{-1}*	Amount Un-spiked of tannic acid µg mL^{-1}*	RSD%**	REC%***
Cinnamon	5.873	15.959	10.073	0.442	100.22
	10.755	20.681		1.037	98.63
	21.841	30.416		0.825	93.14
	30.273	39.976		1.508	98.78
Anise	5.873	19.912	14.202	0.879	97.22
	10.755	24.639		0.718	97.04
	21.841	34.384		1.769	92.40
	30.273	43.726		1.569	97.53
rosemary	5.873	41.768	36.459	0.269	90.39
	10.755	46.829		0.523	96.42
	21.841	56.532		0.591	91.91
	30.273	66.035		0.387	97.69

* Average of 3 determinations. **RSD%=(SD/mean) *100%
***recovery%= (amount of spiked sample-amount of un-spiked sample/amount added) *100% [26]

TABLE 9 Comparison between the current procedure and reported procedure for the determination of tannic acid

Method	matrix	λ$_{max}$ nm	Linearity	LOD	LOQ	r²/r	RSD%	Ref
HPLC-C18 column (250 × 4.6 mm ,5 µm)	Quercus Species	270	-	1.50 ppm	4.95 ppm	r²=0.999	-	[27]
Nucleosil column 100-5 C18; 250 mm length, 4.6 mm inner diameter, 5 µ particle diameter	Penicillium spinulosum	254	1.04×10^{-5} - 8.32×10^{-5} M	2.2×10^{-6} M	6.6×10^{-6} M	-	-	[28]
High Performance Thin Layer Chromatography	Bryophyllum pinnatum	270	20-100 µg mL^{-1}	19.21 ng	77.48 ng	r²=0.997	-	[29]
HPLC-C18 5 µm particle size,25 cm × 3.2 mm	Pueraria tuberosa (Fabaceae)	270	0-60 µg/mL	-	-	r²=0.997	1.168	[5]
HPLC- C18 (4.6 mm × 150 mm, 5 µ particle size)	Carica papaya leaf	276	20-50 µg/mL	1 µg/mL	5 µg/mL	r=0.999	-	[1]

column ODS-II (4.6 mm i.d. × 250 mm)	Rat	210	50-1000 µg	-	-	r^2=0.9997	-	[30]
C18 column (250 × 4.6 mm i.d.; 5 µm)	herbal drugs	250	-	-	-	-	4.9%	[31]
HPLC- a column (250 mm×4.6 mm, 5 µm 100-5 C18ec)	Cinnamon, anise, rosemary	277	0.1-60 µg mL^{-1}	0.0037 µg mL^{-1}	0.0122 µg mL^{-1}	0.9989	6.118	This work

Conclusion

The proposed method is simple, rapid, and economical and was conducted using new conditions for the determination of tannic acid by high-performance liquid chromatography, with column (250mm×4.6 mm,5µm) C18 at a flow rate of 1.5 mL/min and retention time for tannic acid was 1.6 at pH 6. These conditions were successfully applied to the extracts of the herbs, which are rosemary, anise, and cinnamon, and showed good estimated amounts. This method also showed good performance, selectivity, linearity, repeatability, accuracy, and specificity, in addition to good LOD and LOQ.

Acknowledgements

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