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Quantification of catechin in *Acacia catechu* extract by non-derivative, first derivative UV/Vis spectrophotometry and FT-IR spectroscopy

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The aim of the present study was to quantify catechin in spray-dried extract of *Acacia catechu* by applying non-derivative (ND), first derivative (FD) UV/Vis spectrophotometry and FT-IR spectroscopy. The ND methodology at pH = 7.9 demonstrated to be the most sensitive, linear, precise, simple and accurate among all applied methods. Catechin content in two series of 12 *Acacia catechu* extract solutions (70% EtOH) at pH = 4.0 and pH = 7.9, respectively, was determined by the developed UV/Vis ND methods. The statistical analyses between the experimental data sets obtained by both techniques proved to be statistically significant. The highest catechin content in the non-diluted ethanol *Acacia catechu* extract was quantified as 169.88 mg/L at pH = 7.9 and 171.52 mg/L at pH = 4.0. The comparative analyses of the FT-IR spectra of pure catechin and *Acacia catechu* extract in powdered form and the insignificant bands width and intensity deviations proved undoubtedly the high content of the natural antioxidant in the plant extract. The latter conclusion was sustained by the established significant average percent recovery (97.17%) of catechin in the raw plant extract.

Keywords: *Acacia catechu*, catechin, UV/Vis, FT-IR

INTRODUCTION

Various natural antioxidants are being used to neutralize the harmful effects of reactive oxygen species (ROS) overproduced in diseased tissues and contaminated environments. Catechins are flavan-3-ols that are found widely in medicinal plants and are utilized for anti-inflammatory, antimicrobial, cardio protective, hepato-protective, neural protection, antimalarial [1] and other biomedical applications [2**],**

In view of the growing interest in bioflavanols, including catechins, scientific literature reports variety of methods for their extraction [3], separation and quantification in plant materials and food. Qualitative and quantitative determination of catechins has been significantly facilitated by HPLC techniques applying various detectors: UV, PDA [4], DAD, fluorescence, electrochemical, LC-MS, etc. [5,6]. Near-infrared spectroscopy, TLC and GC-MS have also been used for catechins quantification [7-10].

In this context, UV/Vis spectrophotometry is characterized as a simple technique with low operating costs giving fast and reliable results for polyphenols determination [11-13]. Among the main limitations, however, is the necessity of time consuming pre-separation techniques for interferences removal, as well as the low selectivity. In this respect, derivative UV/Vis spectrophotometry has been established as a vehicle to overcome such analytical problems encountered with conventional spectrophotometry, as it allows spectral interferences removal, increases assay selectivity and specificity, reduces noise and improves signal amplification [14]. Besides, the study of Zhou *et al.*, (2018) established the high feasibility of FT-IR spectroscopy for efficient detection of catechin monomers and caffeine in fresh tea leaves [15, 16]. In their study Park *et al*., (2015) assessed quantitatively the main extracted polyphenols from commonly consumed fruit and their antioxidant activities by FT-IR spectroscopy and proved the method suitability for bioactivity determination [17]. The results obtained by UV/Vis and FT-IR analyses on catechins/epicatechins contents and on the bactericidal activity of nanoparticles present in green tea extracts displayed high correlation, which indicated the applicability of both methods for polyphenols determination [13].

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In this respect, the present study was provoked by the valuable pharmacological properties and biomedical activities (antimicrobial, anti-inflammatory, antifungal, coagulant, vermifuge, antidiarrheal, etc.) of *Acacia catechu*, a plant indigenous in India, other Asian countries, and East Africa [18], and by the fact that currently there are no scientific reports on UV/Vis, derivative UV/Vis spectrophotometry and FT-IR methods for catechin quantification in *Acacia catechu* extract. Therefore, the aim was to quantify catechin in spray-dried extract of *Acacia catechu* by applying non-derivative (ND), first derivative (FD) UV/Vis spectrophotometry and FT-IR spectroscopy.

EXPERIMENTAL

Chemicals

(+)-Catechin hydrate (≥ 96.0 %, HPLC), C_2H_5OH (≥ 99.8 %, HPLC), CH_3COOH and NaOH (p.a., HPLC) were supplied by Sigma-Aldrich. *Acacia catechu* spray-dried extract was supplied from Northern India.

UV/Vis spectrophotometric analyzes

A catechin hydrate standard solution in 70% EtOH with initial concentration of 200 mg/L was prepared. Two series of 12 ethanolic catechin hydrate solutions each within the concentration range C_o 1 – 200 mg/L were prepared by the dilution method at pH = 4.0 and pH = 7.9, respectively. Two series of 12 ethanolic extract solutions at initial pH = 4.0 and pH = 7.9, respectively, were prepared by dissolving different quantities of the dried extract (1-20 μg) in 10 mL of 70% EtOH solutions. The pH of the standard and extract containing ethanol solutions was adjusted to pH = 4.0 by the addition of 2M CH_3COOH.

Catechin concentrations in EtOH were measured on a UV/Vis spectrophotometer DR 5000 Hach Lange (Germany), supplied with 10 mm quartz cells. All spectra were recorded in the UV region at λ = 281 nm at pH = 7.9 and pH = 4.0 with 2 nm slit width, 900 nm min^{-1} scan speed and very high smoothing.

FT-IR spectroscopy

FT-IR spectrum of the dry powdered *Catechu Acacia* extract was determined on Bruker Tensor 37 FT-IR spectrometer using KBr pellet technique. For the sample, 64 scans were collected at a resolution of 2 cm^{-1} over the 4000–400 cm^{-1} wavenumber region.

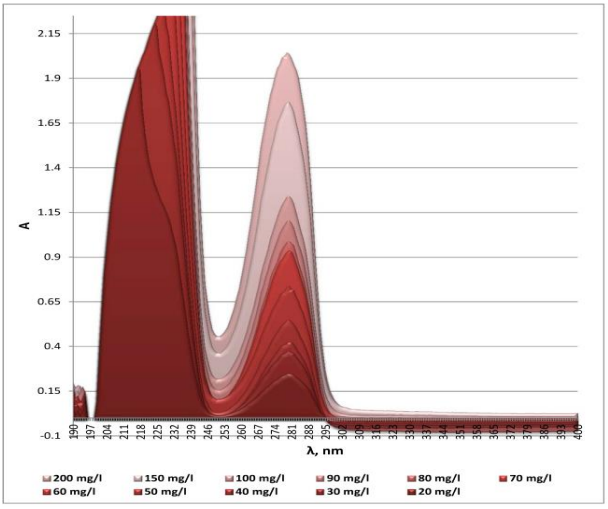
Statistical and Error Analysis

All experiments were carried out in triplicate, and the average values were taken to minimize random error. The values of the error criteria functions R^2, mean squared error (MSE), root mean square error (RMSE), mean absolute percentage error (MAPE), Durbin Watson (DW) function and Akaike information criterion (AIC) were determined by XLStat for Excel linear regression analyses.

RESULTS AND DISCUSSION

UV/Vis spectrophotometric analyses of standard (+)-catechin hydrate

The UV/Vis spectra of catechin hydrate ethanolic solutions in slightly alkaline medium (pH = 7.9) (Fig. 1) and acidic medium (pH = 4.0) (Fig. 2) were well resolved and displayed maximum absorbance in the UV region at λ 280 nm for the entire concentration range of 10 – 200 mg/L.



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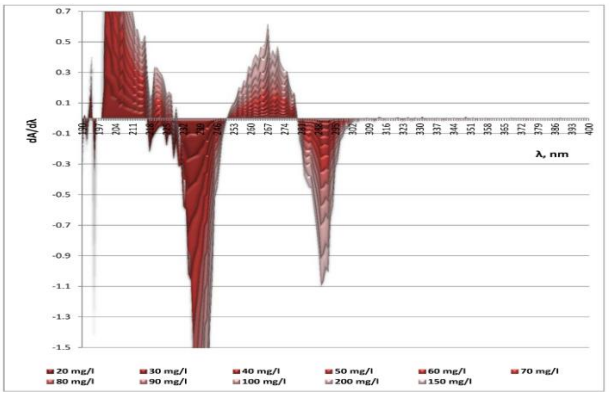

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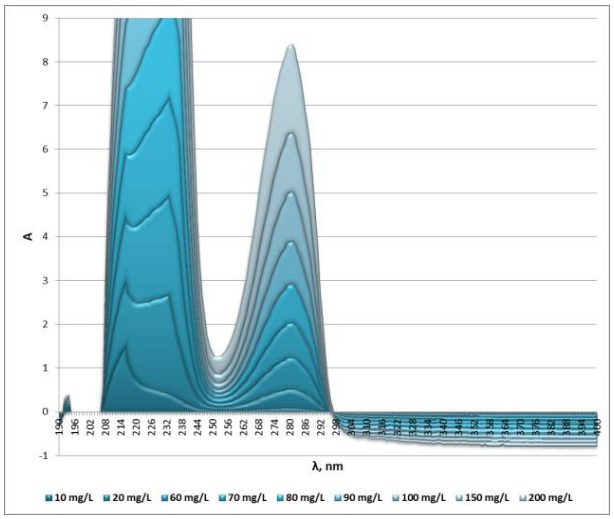
Fig. 1. **A**. Non-derivative and **B**. Derivative UV/Vis spectra of catechin hydrate solutions in 70% EtOH at pH = 7.9 (λ = 280 nm).



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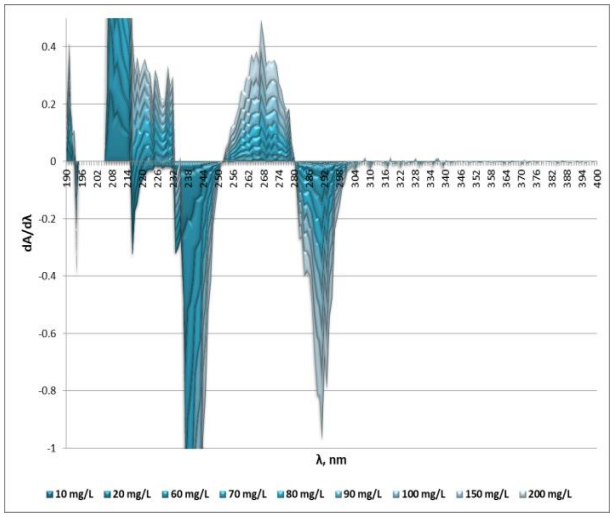

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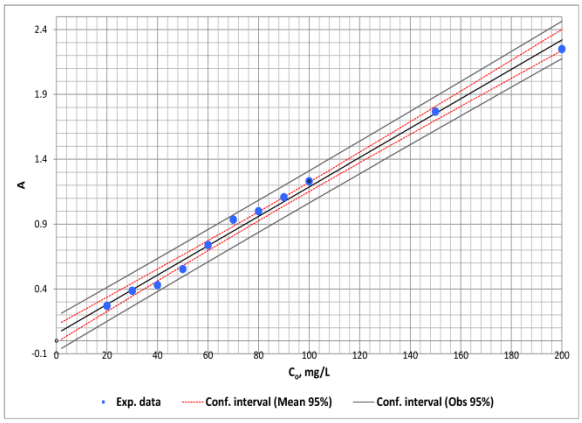
Fig. 2. **A**. Non-derivative and **B**. Derivative UV/Vis spectra of catechin hydrate solutions in 70% EtOH at pH = 4.0 (λ = 280 nm).

The obtained standard curves of catechin hydrate (Fig. 3) are characterized with satisfactory linearity and accuracy as observed by the significantly low values of the regression coefficient (R^2), mean squared error (MSE), root mean square error (RMSE) and Akaike information criterion (AIC) (Table 1).

The Durbin Watson (DW) function is a test for autocorrelation in the residuals from a statistical regression analysis. The calculated DW value is within the interval (0; 2), which is indicative for positive autocorrelation between the concentrations data set. The mean absolute percentage error (MAPE) is a measure of prediction accuracy of a forecasting method in statistics. However, it could also be used as a loss function for regression problems in analytical chemistry. The MAPE value < 10 calculated in the present study is interpretative of the high accuracy of the analytical results.



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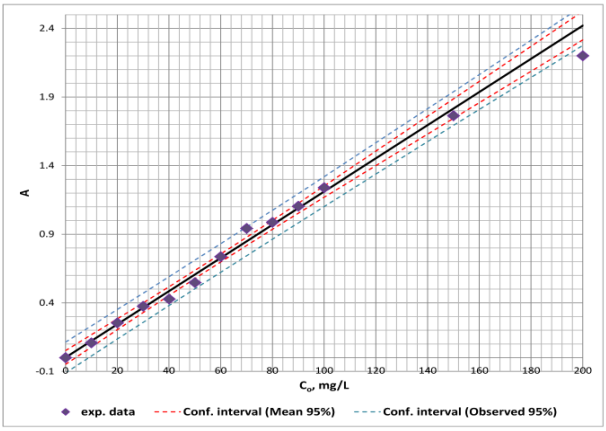

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Fig. 3. UV/Vis calibration curves of catechin hydrate at pH = 7.9 at λ = 280 nm: A. Non-derivative, **B**. Derivative UV/Vis spectrophotometry.

The developed UV/Vis methods at pH = 7.9 and pH = 4.0 employed standard catechin hydrate ethanol solutions. Based on the analyses of the experimental data it was established that the ND spectrophotometric techniques are characterized with lower LOD and LOQ values as compared to the FD methods, despite of the more favorable statistical error criteria analyses. Besides, the ND methodology at pH = 7.9 demonstrated to be the most sensitive, linear, precise, simple and accurate among all applied methods.

UV/Vis spectrophotometric analyses of Acacia catechu extract

To determine the concentration of catechin in *Acacia catechu* extract a stock solution was prepared by dissolving 20 mg of dry extract powder in 100 mL of 70% EtOH. Two series of 12 samples each were prepared by dilution of the stock extract-containing solution with 70% EtOH, at dilution ratios presented in Table 2, to a final volume of 10 mL.

Table 1. Values of error functions/criteria, lower limit of detection (LOD) and lower limit of quantification (LOQ) of catechin hydrate according to UV/Vis spectrophotometric analyses.

UV/Vis method	Non-derivative pH = 7.9	Non-derivative pH = 4.0	Derivative pH = 7.9	Derivative pH = 4.0
		Linear equation		
Error function	$A = 5.4055 \cdot 10^{-2} + 0.0113 \cdot C_o$	$A = 4.933 \cdot 10^{-3} + 9.8856 \cdot 10^{-3} \cdot C_o$	$A = 1.19 \cdot 10^{-3} + 1.2094 \cdot 10^{-2} \cdot C_o$	$A = 4.9632 \cdot 10^{-3} + 9.8175 \cdot 10^{-3} \cdot C_o$
R^2	0.993	0.982	0.993	0.983
MSE	0.003	0.006	0.002	0.006
RMSE	0.053	0.080	0.045	0.077
MAPE	5.180	13.728	5.789	13.462
DW	1.078	1.869	1.466	1.819
AIC	-68.532	-63.979	-72.602	-64.864
LOD (mg/L)	**6.368**	**11.903**	8.197	13.417
LOQ (mg/L)	**21.227**	**36.07**	24.84	40.657

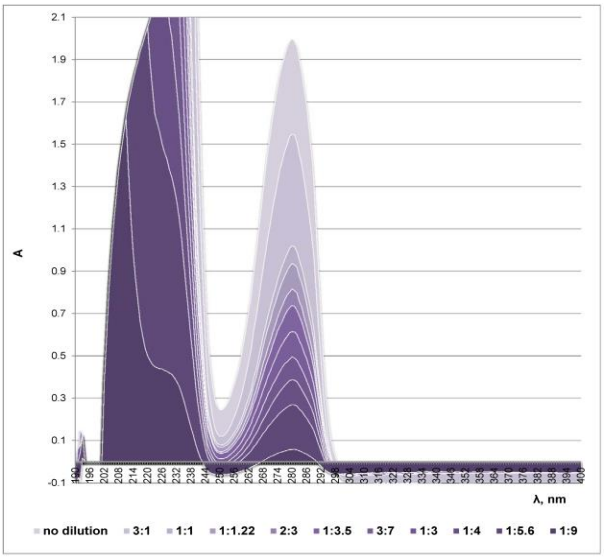
Table 2. Catechin contents and % recovery in *Acacia catechu* extract determined by non-derivative spectrophotometry.

Sample No.	Mixing ratio (v:v)	Extract concentration, mg/L	Abs	Catechin concentration, mg/L	Recovery, %	Abs	Catechin concentration, mg/L	Recovery, %
			pH = 7.9			pH = 4.0		
1	1:19	10	0.06	0.762	7.62	0.1055	10.170	101.70
2	1:9	20	0.158	7.812	39.06	0.237	23.472	117.36
3	1:5.6	30	0.268	17.724	59.08	0.334	33.285	110.95
4	1:4	40	0.385	28.549	71.37	0.3675	36.674	91.69
5	1:3	50	0.490	38.107	76.21	0.439	43.906	87.81
6	3:7	60	0.614	47.635	79.39	0.579	58.068	96.78
7	1:3.5	70	0.734	57.665	82.38	0.726	72.938	104.20
8	2:3	80	0.808	65.216	81.52	0.779	78.300	97.88
9	1:1.22	90	0.931	76.131	84.59	0.8775	88.264	98.07
10	1:1	100	1.015	83.240	83.24	0.9275	93.322	93.32
11	3:1	150	1.587	134.479	89.65	1.1985	120.735	80.49
12	no dilution	200	1.987	169.877	84.94	1.7005	171.516	85.76
					average 69.92%			average 97.17%

The concentrations of polyphenol in the extract series was determined by both ND UV/Vis methods. The UV/Vis spectra of catechin in slightly alkaline and acidic solutions (pH = 4.0) (Fig. 4 A, B) displayed maximum absorbance peaks in the UV region at λ 281 nm for the entire concentration range. The concentrations of the biologically active substance in the extracts and the percent recovery for both experimental series are presented in Table 2.

The highest catechin content in the non-diluted ethanolic *Acacia catechu* extract was quantified as 169.88 mg/L at pH = 7.9 and 171.52 at pH = 4.0. The recovery interval ranged from 80.49% to 117.36%, for the experimental series at pH = 4.0 and from 7.62% to 89.65% - at pH = 7.9. The recovery test measures the amount of the analyte present or added in the analytic portion of the test material that is recovered and could be quantified. The acceptable recovery intervals depend on the analytical complexity and the sample, and can range from 50 to 120% with precision up to ±15% [4, 19].

Obviously, the results obtained in an acidic medium are characterized with higher accuracy. Besides, the latter method is more suitable for scientific investigations with catechin, as it is well known that aqueous catechin solutions are unstable due to rapid oxidation/degradation, while ethanol solutions are characterized with higher stability, which is additionally increased by acidification of the solution [20, 21].



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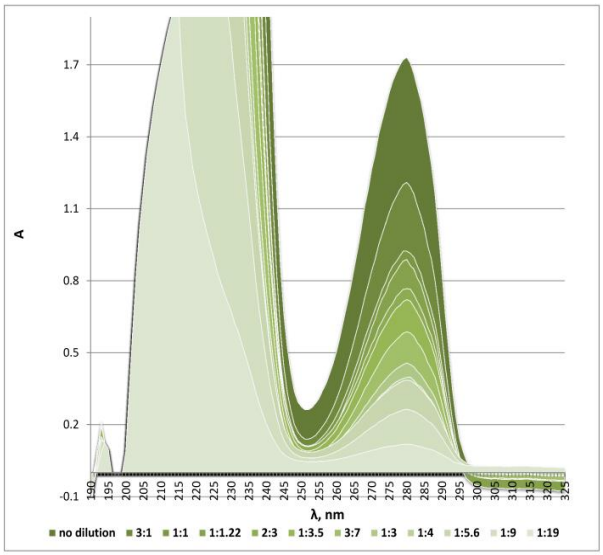

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Fig. 4. UV/Vis spectra of *Acacia catechu* extracts at **A**. pH = 7.9 (λ = 281 nm); **B**. pH = 4.0 (λ = 281 nm).

Regarding the increasing number of investigations on plant polyphenols and their applicability for pharmaceutical, bio-medical and agricultural purposes in the last decade, as well as their encapsulation in various micro- and nano-matrices [22, 23], acetic acid is an appropriate acidifying agent [24]. Moreover, some methodologies such as encapsulation on natural mineral microparticles provoke pH increase during the incorporation process due to the alkaline nature of the supports, which in turn would provoke immediate photooxidation of catechin associated with unwanted color and structural changes influencing negatively the bioactivity of the natural polyphenol.

In this respect the developed accurate ND UV/Vis method in acidic medium will be valuable for quantification of catechin in various plant extracts and other studies subjected to catechin.

FT-IR study of Acacia catechu extract

The FT-IR spectra of catechin standard and *Acacia catechu* dry extract are presented on Fig. 5. The wavelengths of the characteristic bend assignments are given in Table 3.

Table 3. Characteristic bands on FT-IR spectra of catechin hydrate and *Acacia catechu* extract [13,25-27].

Bend assignments	Wavenumber, cm^{-1}	
	Catechin hydrate standard	*Acacia catechu* extract
C-H alkenes	965.3	970.16
-C-O alcohols	1020.1	1031.8
-OH aromatic	1144.1	1149.53
-C-O alcohols	1285.0	1245.97
Coordination bonding of -OH groups of aromatic ring	1364.9	1369.4
C-H bending vibration	1474.4	1475.49
C=C aromatic ring	1514.5	1523.71
C=C alkenes	1610.5	1618.22
coordination bonding of -OH groups of aromatic ring	1864.2	1865.1
methylene ($–CH_2$) C-H stretching	-	2923.9
O-H stretching	3412.0	3404.2; 3255.7

The comparative analyses of the FT-IR data outlined slight variations in the intensity and width of some peaks of the extract as compared to the standard, which could be explained as by the method of extraction so by the presence of a number of other polyphenols, tannins, carboxylic acids, etc. The broad bands at 3404.2 and 3255.7 cm^{-1} are corresponding to OH-groups. The absorption band at 2923.9 cm^{-1} indicates methyl ($-CH_3$) and methylene ($-CH_2$) stretching. The peak at 1523.71 cm^{-1} is indicative of C=C aromatic ring vibrations, while that at 1475.49 cm^{-1} is associated with alkane $-CH_2$ bending vibrations. The bands observed at 1865.1 and 1369.4 cm^{-1} outline coordination bonding of aromatic -OH groups. Peaks at 970.16, 1031.8 and 1245.9, 1149.5 cm^{-1} confirm the presence of alkene C-H, alcohol –C-O and aromatic –OH bending vibrations, respectively.

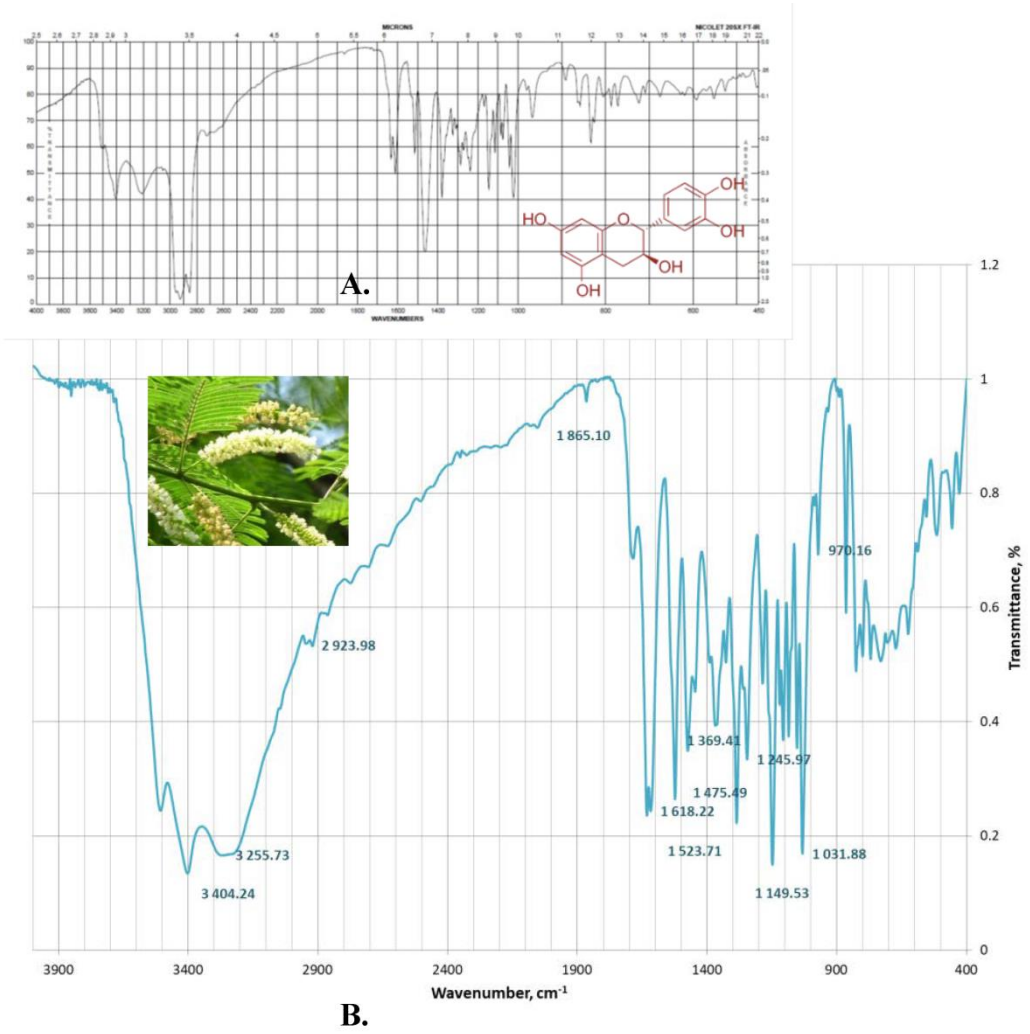

Fig. 5. FT-IR spectra of: **A**. catechin hydrate standard, **B**. Acacia catechu dry extract.

The comparative analyses of the FT-IR spectra of pure catechin and *Acacia catechu* extract in powdered form and the insignificant bands width and intensity deviations proved undoubtedly the high content of the natural antioxidant in the plant extract. The latter conclusion was sustained by the established significant average percent recovery (97.17%) of catechin in the raw plant extract.

CONCLUSIONS

In conclusion, based on the analyses of the experimental data it was established that the ND UV/Vis spectrophotometric techniques are characterized with lower LOD and LOQ values as compared to the FD methods, despite of the more favorable statistical error criteria analyses. Besides, the ND methodology at pH = 7.9 demonstrated to be the most sensitive, linear, precise, simple and accurate among all applied methods. The recovery interval of catechin in *Acacia catechu* extract determined by the ND technique at pH = 4.0 (80.49% - 117.36%) was within the acceptable values and proved the accuracy of the method. The applied UV/Vis and FT-IR assays can be successfully applied as quality and quantity control methods for determination of catechin in ethanol plant extracts.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

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