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RESEARCH ARTICLE

Preliminary identification of Citrullus Colocynthis from Togo by FT-IR and Raman Spectroscopy

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Abstract

Citrullus colocynthys (L.) Schrad. (colocynthis , wild gourd, or bitter apple), is a member of the gourd family Cucurbitaceae. It is a non-hardy, herbaceous perennial vine, branched from the base. Originally from tropical Asia and Africa, it is now widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediterranean region.

Our work consist on preliminary characterization of Citrullus Colcynthis occurs in Togo.

This work aims to solid Raman identification and FT-IR characterization of soxhlet extract with three solvents (Petroleum Ether, Hexane and Dichloromethane).

INTRODUCTION

While infrared (IR) spectroscopy has been well established as a useful tool for structure elucidation and quality control in various industrial applications over more than three decades, Raman spectroscopy was restricted for a long time primarily to academic research. In parallel with the development of Fourier transform (FT) IR, which pushed the usage of both spectroscopic methods dramatically. Accordingly, FT-IR and Raman spectroscopy are complementary techniques for the study of molecular vibrations and structure. The combined techniques results in an analytical method that allows spatially resolved investigation of the chemical composition of heterogeneous foods and food ingredients. Both qualitative and quantitative information can be obtained using Raman/FT-IR spectroscopy. A number of organic compounds and functional groups can be identified by their unique pattern of absorption, and the intensity of the absorption may be used for the calculation of the relative concentration in the sampled entity (Wetzel & LeVine, 1999).Therefore, we choose these techniques for realizing an introductory study of chemical composition of Togo endemic plant (Citrullus Colcynthis).

Materials and Methods

Seed sample collection

Citrullus Colcynthis seeds procured from local stores and markets of Togo via Savanna region in the Nord of Togo (Dapaong, Mango, Kpendjal). This seed were stored 24hours at 40°C. After that seeds were subjected to

mechanical grinding. Powdered seeds were obtained and dried at 37 ^{o}C in an incubator and preserved in clean sealed polyethylene bags at 4 ^{o}C.

Chemicals and Reagents

All chemicals and reagents were from Sigma-Aldrich (Tunisia): Dichloromethane, Hexane and Petroleum Ether .All the chemicals purchased were of analytical grade.

Extraction of oil

The oil was extracted from the kernel by Soxhlet, 20 g of crushed kernel was packed in a thimble and the oil was extracted with three different solvent (Petroleum Ether, Hexane and Dichloromethane) for 6 h. The extract was concentrated using rotary evaporator Stuart RE300DB.

Spectra

ATR-FTIR spectra were recorded on a Nicolet spectrometer. The ATR accessory contained a ZnSe crystal (25 mm×5 mm×2 mm) at a nominal incident angle of 45◦, yielding about 12 internal reflections at the sample surface. All spectra (100 scans at 4.0 cm−1 resolution and ratioed to the appropriate background spectra) were recorded at 25◦C. A special dry system was constructed to prevent interference of atmospheric moisture with the spectra.

Raman microprobe measurements, single spectra (MRS) and images were performed using a Jobin-Yvon Horiba LabRam-HR (high resolution) system interfaced with an Olympus BX41 optical microscope. The system was also equipped with automated x–y micro-sampling stage, 1200 grooves/mm diffraction grating, and a Peltiercooled charge-coupled device (CCD) detector. Spectra were excited using the 632.8 nm emission line of a He–Ne laser. An Olympus ×100 objective (numerical aperture 0.95) was used.

Results and discussion

Proteins and amino acids

Several modes of vibration (FT-IR and Raman) are practical for the interpretation of various amino acids and proteins which occurs in the plant tissue. Most characteristic bands are correlated with the CONH group, referred to as amide A, amide B and amide I–VII [1].

The coming three signals are of main interest for the identification of different protein backbone validation: amide I to be determined between 1680 and 1600 cm^{-1} (stretching vibration of C=O), amide II observed in the range among 1580 and 1480 cm^{-1} and amide III to be found among 1300 and 1230 cm^{-1} (both associated with coupled C–N stretching and N–H bending vibrations of the peptide group). Table 1 summarizes the different vibrations, in Raman spectroscopy, the functional groups of proteins and amino acids.

Additionally the amino acids and proteins were picked out by IR spectroscopy .In this context, the possibility of determining the distribution of lysine in the barley has been described [2]. The functional groups of specific amino acids such that the S-S and the S-H of cysteine and cysteine, and the aromatic rings of tryptophan and the imidazole nucleus phenylalanine and histidine were also performed by IR and Raman spectra currently signed. Identification of compounds containing disulfide bonds can be agreeably obtained by using FT-Raman spectroscopy, because the S–S stretching band is polarized and prominent in the Raman spectra while the IR intensity is usually very week due to its nonpolar nature [3] (Figure 1,Figure 2). Besides, the conformational study of the disulfide bridge can be performed. As could be seen in the Raman spectra of rice globulin, the disulfide bonds of cysteine residues occur in three different conformations [4]. On the one hand, sulfhydryl groups show intensive S–H stretching modes in the Raman spectra to be seen in the regions between 2550 and 2580 cm^{-1}. On the other hand, the major conformation was gauche–gauche–gauche as indicated by the Raman band at 512 cm^{-1}, which is the most preferred conformation in many proteins with –S -S– bridges [5]. Finally, the other minor bands seen at 525 and 540 cm^{-1} were assigned to gauche–gauche–trans and trans–gauche–trans conformation, respectively (Table 1).

Carbohydrates

Sugars were identified by Raman spectroscopy with distinctive bands at 1462, 1126, 840 cm^{-1} and are shown in Table 1 and figure 1.

Lipids and fatty acids

It is known today that the potential of the infrared and Raman spectroscopy is used for the observing of alteration for chemical screening. Furthermore, the boon of Fourier transform technique and new sample presentation techniques such as total reflectance and photoacoustic detection have led to a widespread application of various IR methods in the area of vegetable oil analysis [6, 7].

It was found that the frequency of concrete absorption bands in the fingerprint region (700–1500 cm^{-1}) gives direct information about the ratio among saturated and cis monounsaturated fatty acid acyl groups. We also observed the elongations of vibrations of the double Trans and cis olefinic bond in the region of 3025 and 3006 cm^{-1}, respectively. Also that the stretching vibration between 3000 and 2850 cm^{-1} and the C = O triglyceride vibrates to 1746 cm^{-1}. A

small shoulder to be seen at 1711 cm^{-1} is assigned to a small amount of free fatty acids. The Raman spectrum showed the presence of a characteristic band of the double bond C= C stretching vibration at 1600 cm^{-1}.

Table 1 Assignment for the most characteristic FT-Raman and FT-IR bands of some primary metabolites of Citrullus Colcynthis: Proteins, amino acids, Carbohydrates, Lipids and fatty acids.

Chemical Groups	Representatives	FT-Raman (cm^{-1})	Assignment	ATR-IR (cm^{-1})	Assignment
Amino acids	Cysteine	513	υ (S–S) g–g–g	*	*
		523	υ (S–S) g–g–t		
		540	υ (S–S) t–g–t		
	Methionine	630–670	υ (C–S) g		
		700–745	υ (C–S) t		
		2550–2580	υ (S–H)		
	Tyrosine	850	Fermi resonance of ring fundamental	*	*
	Tryptophan	1360	and overtone		
		878			
		765	δ(ring)		
	Phenylalanine	1004	δ(ring)	*	*
	Aspartic and	1400–1430		*	*
	glutamic acids	1700–1750	υ (C=O)O^-		
Proteins	α-Helix	1650	υ (C=O)OH	1655	Amide I**
		1280	Amide I**		
	Anti-parallel β-sheet	1670	Amide III***	1670	Amide I
		1235	Amide I		
			Amide III		
	Disordered structure	1665		1665	Amide I
			Amide I		
	Solvated	1245			
	Disordered structure	1685			Amide I
			Amide III	1685	
			Amide I		
	(non-hydrogen bonded)	1235			
			Amide III		Amide II****
Lipids/fatty acids				1543–1480	
		3008			
			$υ_{as}$(=C–H)		
		2970			
		2940			
		2885	$υ_{as}(CH_3)$		
		2850	$υ_{as}(CH_2)$		
		1750	$υ_{as}(CH_3)$		υ (C=O)
		1670	$υ_{sy}(CH_2)$	1750	υ (C=C) trans
		1660	υ (C=O)	1670	υ (C=C) cis
		1445	υ (C=C) trans	1660	$δ(CH_2)$
Monosaccharides		1100–800	υ (C=C) cis	1445	
			$δ(CH_2)$		
			υ (C–C)		
	α-Glucose	847			
			(C–O–C)		
	β-Glucose	898	skeletal mode (C–O–C)		

Disaccharides	β -Fructose	868	skeletal mode (C–O–C)	1126	υ (C–O)
	Sucrose	1462 847	skeletal mode δ(CH2)		
	Maltose	847	(C–O–C) skeletal mode		
	Cellobiose	885	(C–O–C) skeletal mode		

Abbreviations: as: asymmetric, sy: symmetric Vibrations— υ: stretching; δ: deformation; (*) no data; (**) pure standard.

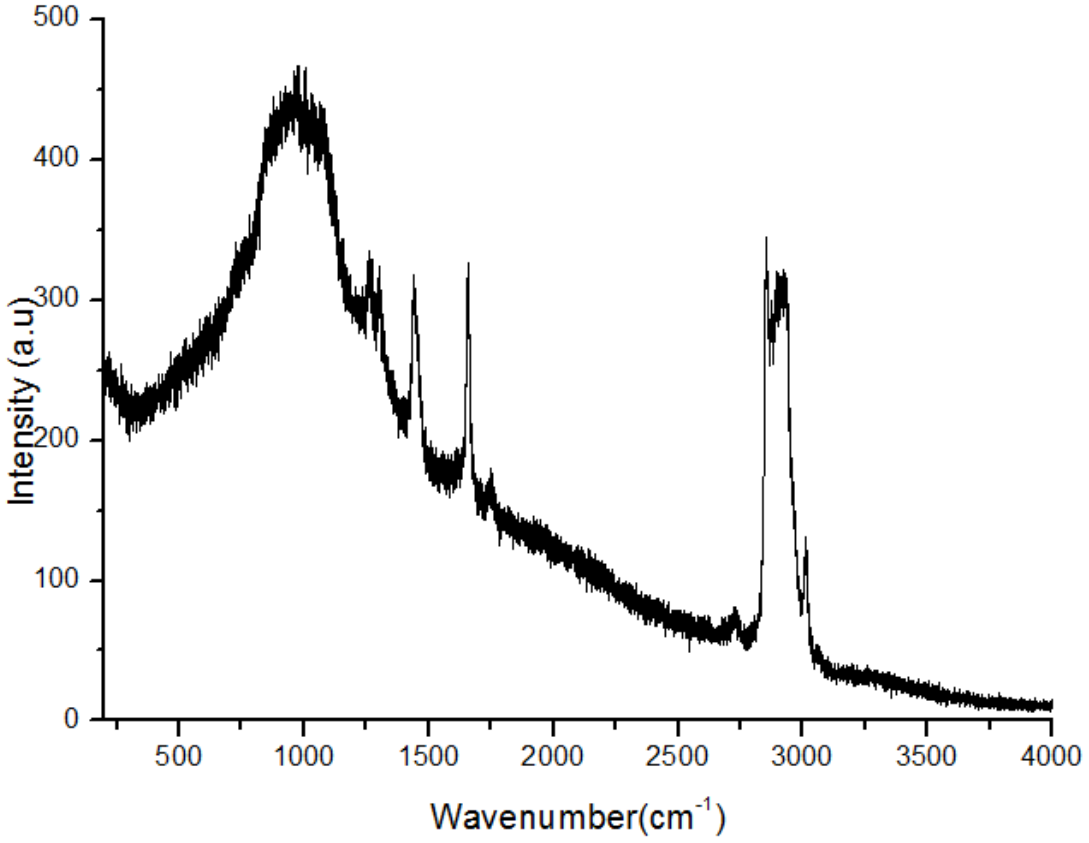

Figure 1: Raman spectrum of Citrullus Colcynthis

Phenolic substances

Flavonoids

These are phenolic compounds with two aromatic rings bonded by a C3 unit (central pyran ring) and may be divided in several classes based on the oxidation state of the pyran ring and on the characteristic color, e.g.: flavones, flavonols, flavonol glycosides, flavanones, flavanone glycosides, anthocyanins, flavanols (catechins) and chalcones [8].

In the present working, the spectral range among 500 and 900 cm^{-1} strongly depends on the glycosylation pattern. Anthocyanidin monoglycosides exhibit a strong RR signal close to 540 cm^{-1} while 3, 5-diglycosides have strongest

feature in the lower frequency range close to 630 cm^{-1}. Additionally, both types of glycosides differ also in the relative intensity and in the shape of two lines located at 1645 and 1350 cm^{-1} in Raman spectroscopy.

Alkaloids

Alkaloids represent a group of nitrogen-containing bases and most of them are used for medicinal purposes. But, the FT-Raman spectra obtained from Citrullus Colcynthis show significant key signals of piperine [9].

Apart from the intense –C–H stretching vibrations between 2800 and 3100 cm^{-1}, the main Raman signals occur in the fingerprint range among 1100 and 1630 cm^{-1}. Additionally, the aromatic and aliphatic –C–C– as well as N–C =O stretching vibrations can be detected among 1580 and 1635 cm^{-1}.

Also, the signals observed at 1448 cm^{-1} can be assigned to $–CH_2$ bending vibrations, whereas the other bands in the range among 1100 and 1400 cm^{-1} are mainly due to –C–C– stretching (1153 cm^{-1}) as well as $–CH_2$ twisting and rocking vibrations (1295 and 1256 cm^{-1}) of piperine molecules. While, the corresponding IR spectrum several specific piperine signals, e.g. due to =C–O stretching vibrations at 1194 and 1252 cm^{-1} as well as wagging vibrations at 996 cm^{-1}. Raman spectra in the fingerprint range among 700 and 1500 cm^{-1} show numerous sharp bands which are mainly assigned to deformation and stretching vibrations of the alkaloid ring system. As well as, the strongest IR bands, which are predominantly due to –C–O–C– stretching modes can be found in the 1050 cm^{-1} region.

Terpenoids

The terpenes are built from isoprenoid units with the general formula $(C_5H_8)_n$. According to the amount of isoprenoid units (n) they can be divided into several classes: hemiterpenes (C_5H_8), monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), triterpenes ($C_{30}H_{48}$), tetraterpenes ($C_{40}H_{64}$) and polyterpenes $(C_5H_8)_n$ [10]. The terpenes were also characterized by Raman and FT-IR spectroscopy. Then, in our work, terpenes characteristic bands are presented in Table 2 and figure 2.

- **Monoterpenes ($C_{10}H_{16}$)**

Monoterpenes are the most abundant group of terpenoids and they built two isoprenoid units. This monoterpenes can be divided into three groups, acyclic terpenes found, for example, monocyclic and bicyclic. However, acyclic monoterpenes show the most intense bands due to stretching vibrations of C=C bonds at about 1670 cm^{-1} in the Raman spectrum, whereas FT-IR spectra are more miscellaneous. Terpene (Monocyclic and Bicyclic) give bands due to C-H vibrations between 800 and 920 cm^{-1} in FT-IR spectroscopy. However, using Raman spectroscopy differentiation among these groups is clearer. Ring deformation vibration observed in the FT-Raman spectrum of monocycles between 740 and 760 cm^{-1} and can be therefore recognized in the range among 645 and 666 cm^{-1}.

In the FT-Raman spectrum characteristic C=C stretching vibrations appear at 1611 cm^{-1} for α-terpinene and at 1701 cm^{-1} for γ-terpinene reflecting the difference among a conjugated and a nonconjugated system, respectively. In ATR-IR spectroscopy γ-terpinene can be identified by CH and CH_2 wagging vibrations at 781 and 947 cm^{-1}, whereas α-terpinene shows only one intensive signal at 823 cm^{-1}. Finally, among monoterpenes numerous alcoholic derivatives can be well recognized by IR spectroscopy where the intense IR band, due to the out-of-phase C–C–O stretching mode, is seen for primary alcohols at 1075– 1000 cm^{-1}.

- **Sesquiterpenes ($C_{15}H_{24}$)**

Sesquiterpenes are used as antifungal, carminative or antibiotics. Sesquiterpenes found in chamomile (Matricaria recutita L.), such as a-bisabolol and b-bisabolol are known for their anti-inflammatory activity [9]. Table 2 and figure 2 shows the spectroscopic characteristics for some sesquiterpenes.

- **Tetraterpenes ($C_{40}H_{64}$)**

FT-Raman spectroscopy also gives a strong enhancement of carotenoids due to the known preresonance effect; furthermore disturbing fluorescence effect of biological material usually observed when laser excitation is performed in the visible range, are avoided [11]. Schulz et al. have shown that the wave number location of these bands, and in particular the υ_1 band, is strongly dependent on the length of the carotenoid chain, and generally, carotenoids with 11, 9, 8, 7 conjugated C=C bonds have their characteristic bands at about 1510, 1524, 1530, 1536 cm^{-1} [12,13].

Strong bands of carotenoids are observed in the Raman spectrum within the 1500–1550 cm^{-1} and 1150–1170 cm^{-1} ranges due to in-phase C=C (υ_1) and C–C stretching (υ_2) vibrations of the polyene chain. Additionally, in-plane rocking mode of CH_3 groups attached to the polyene chain and coupled with C–C bonds are seen as a peak of medium intensity in the 1000–1020 cm^{-1} region.

Table 2: Assignment for the most characteristic FT-Raman and FT-IR bands of some primary metabolites: Terpenoids, Alkaloids and Phenolic substances.

Chemical	Representatives	FT-Raman	Assignment	ATR-IR	Assignment

Groups		(cm^{-1})		(cm^{-1})	
Terpenoids	Monocyclic	1613	υ(ring)		
	Monoterpenes	1208	δ(ring)	1515	
		804	δ(ring)	813	ω (C–H)
	Bicyclic	652	δ(ring)	1374	δsy(CH3(CO))
	Monoterpenes			1214	υ as(C–O–C)
				1079	υ s(C–O–C)
				984	ω (CH2)
				843	ω (C–H)
	Sesquiterpenes				
		1677	υ (C=C)	1437	δ (CH_2)
		1436	δ (CH_2)	1375	$\delta_{sy}(CH_3)$
		1380	δ sy(CH_3)	828	ω (CH_2)
Alkaloids	Tetraterpenes				
		1536	υ (C=C)	*	
		1165	υ (C=C)	*	
Phenolic substances		1100	υ (C–H)		
		1580	υ aliphatic –C–C–		
		1635	N–C =O		
		1448	δ (CH_2)		
		1440	υ (C=C)	1473	υ (C=C)
		1650	υ (C=O)	1750	υ (C=O)

Abbreviations: Vibrations— υ: stretching; δ: deformation; ω: wagging;; (*) no data.

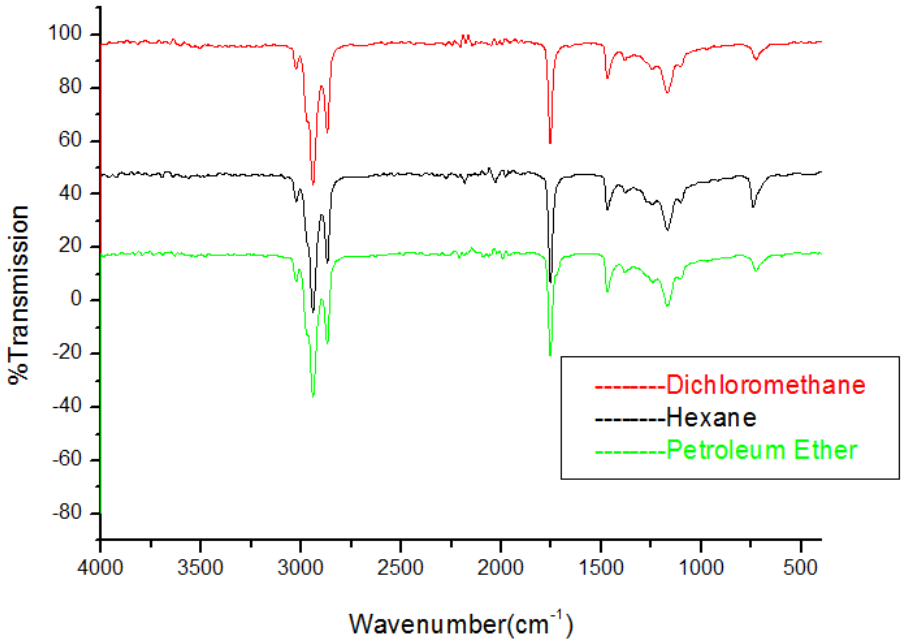

Figure 2: FT-IR analysis of Essential oils

Conclusion

Both Raman and FT-IR micro spectroscopy offer information on the molecular vibrations and structure of food samples. Raman has an advantage because of its ease of sampling, its higher resolution and the possibility for confocal measurements, but the lower signal to noise ratio, the risk of damaging the sample with the laser, and especially auto fluorescence of the sample may hamper its applicability and provide an option for FTIR micro spectroscopy.

This study demonstrate that the Togolienne Citrilus Colcynthis used as a food is a source of a huge number of bioactive compounds such Proteins and amino acids,Carbohydrates,Lipids and fatty acids, Phenolic substances, Alkaloids and Terpenoids.

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