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Experimental approach: FTIR and ^{1}HNMR based metabolic fingerprinting detect drugs interactions with physiological pathways of incubated edible plants in acidic medium

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Abstract

The objective of the present study was investigating impacts of popular drugs on the metabolic fingerprinting of two edible plants, D. *carota* root and P. *sativum* leaves under synthetic stomach conditions regarding temperature and pH. Spectral analyses of fourier transform infrared (FTIR) and nuclear magnetic resonance (^{1}HNMR) of incubated plants with 1/24 part of each tablet in water "pH 5" showed pathways fluctuation within 90 minutes. FTIR spectra of incubated plants in the painkiller, hypersensitive and the drugs mixture showed fatty acids overall shifted spectra. The hypersensitive had influenced the saccharides, polypeptides and proteins. Calcium, vitamins and the painkiller induced polypeptides, proteins and saccharides variations. The 1HNMR spectra of incubated plants with the hypersensitive have detected disappearance of total sixteen metabolites, and the appearance of eleven ones in both plants. The ^{1}HNMR spectra of painkiller plants have detected eighteen metabolic absence and twelve new appearance of metabolites. We hypothesize the possible potential role of drugs in reacting with vegetables physiological pathways in the human stomach, starting with forming new complexes and ending with shifting the metabolic fingerprinting of plants. However, the subsequent impact of these originated complexes on human health with the accumulated practice of eating vegetables together with medicine requires further studies

Key words : *Daucus carota*, Drugs, Fourier transform infrared; FTIR, Nuclear resonance of the proton; 1H NMR, *Petroselinum sativum.*

INTRODUCTION

Globally, *P.sativum* and *D. carota* are largely consumed as food or fodder. For health sakes, molecular and metaboliccomposition; the so called: "authenticity" of vegetables should be preserved even under high medications. It has been thoroughlystudied that food presence in the stomach during drug therapy became an obstacle for the proper action of medicines[1,2,3].

Nonetheless, a possible scenario of a developed interference of these drugs with the metabolic fingerprinting of vegetables inside the stomach has not yet investigated. The FTIR technique has so far became a concise technique to identify biomolecules and distinguishing them from airborne molecules. Recently, FTIR became the potent method in characterizing plants genera from each other- the job difficult for the ordinary microscopes to accomplish [4]. Besides Its efficiency regarding identification of pollen grains[5]. Technologically, FTIR spectroscopy became a fingerprinting technique, discriminates between citrus lines based on their sugars and acidic contents [6]. It has been exploitedin detecting biochemical changes in some pathogenic fungi following treatments [7]. Added to its importance in conveying information about protein changes and enzyme action, identifying drugs mode of action and as a signature for anticancer drugs[8].As such, metabolomic fingerprinting has so far applied in broad fields of researches concerning medicinal plants [9, 10], and in the discovery and quality of herbal drugs [11, 12].The NMR (nuclear magnetic resonance) is another powerful technique, been largely adopted for metabolites fingerprinting detecting spectroscopically every minor change in the chemical structure, and hence predicting the physiological behavior ofplants. The NMR became potent regarding the metabolomics approach in discriminating the authentic product from others[13]. Subsequently, ^{1}HNMR metabolic profiling was a preliminary tool used to prove cases of authentication oradulteration in plants and food, for example, disclosing cases of contamination of saffron with other plants parts [14]. It was also used to discriminate soil lipids from plants lipids [15]. In the present investigation, we devoted our concern to analyze -using FTIR and ^{1}HNMR techniques the metabolic profile and the physiological pathways shifts of plantsfollowing 90 minutes of incubation with the drugs: hypersensitive, painkiller, vitamins, calcium, and their mixture in acidic water(pH 5.0). The purpose of the present study wasverifying the physiological and biochemical instabilities in some edible plantsafter incubation with popular drugs in an acidic media like stomach's acidity. We highlighted our data to be a beginning offurther useful discussions concerning the fact of interference of drugs and complexes originated in a faded stomach with vegetable plants from family: Apiaceae.

MATERIALS AND METHODS

Plants and experimental preparations

Leaves and roots were washed thoroughly with distilled water. Leaves from *P. sativum* were cut to small pieces and *D. carota*roots were cut to small cubes using a cutting razor.

Preparation of acidic water (pH 5.0)

The acidic water was performed as follow: In 300 ml beaker, 150 ml HCl (0.1N) was mixed with distilled water. The acidity was adjusted using NaOH pellets at pH 5.0. The volume was completed to 250 ml with distilled water, covered with cellophane membrane and stored at 25° C. Six beakers were prepared similarly. Each value was the mean of three variables.

Drugs solutions and plants incubation

Hypersensitive, painkiller, vitamins and calcium drugs were purchased from a pharmacy. Each tablet was mixed with 24 ml of

acidic water "pH 5". One ml of each tablet solution was added to the readily prepared acidic water, mixed well and covered using cellophane membrane. The first beaker, designated the control and was tablets free. The second beaker, contained calcium solution "1 ml", the third, contained 1ml of vitamins solution, the fourth, contained 1 ml of the hypersensitive solution, the fifth contained 1ml of the painkiller solution, and the sixth beaker contained 1ml of five tablets mixture. The pH was determined in each beaker immediately after tablets addition.

In each beaker including the control, 20 gm from *P. sativum* leaves and 20 gm of *D. carota* roots were immersed, covered with cellophane membrane, and incubated at 37° C in laboratory water bath for 90 minutes. At the end of the incubation period, the pH was again measured in each case. The samples were washed using distilled water, dried between cheese clothes, and oven dried at 50 °C for 48 h till complete dehydration in laboratory oven. The plant material was ground to fine powder using electrical blender then divided into two sets for both ^{1}HNMR and FTIR analyses. The first set, was saved for FTIR and the second set was extracted in $CDCl_3$and filtered using Whattman no.1. The extracts were collected and preserved at 4° C and saved for ^{1}HNMR analysis.

FTIR spectral analyses

Aliquots of fine plant powder were mixed with KBr pellet, then the spectral transmittance was depicted in the wavenumber range from 4000 till 400 cm-1. The FTIR spectra was obtained from a tensor 27 (Bruker Optics GmbH, Ettlingen, Germany), analyzed by DTGS (deuterated tri-glycine sulfate). Aliquots of 5µl sample was taken to 384-well ZnSe plate and dried on 37°C plate for 20 min. FTIR were measured using a Tensor 27 equipped with a high efficiency HTS-XT automation device. The spectra of each sample was the average of a repeated run in the range 4000-400 cm-1 and with 4 cm-1 interval of spectral resolution.

^{1}HNMR spectral analyses

The ^{1}HNMR spectra of $CDCl_3$ extracts were recorded on Bruker AVANCE 600 spectrometer (Bruker Biospin GmbH, Rheinslettern, Karlsluhe, Germany), operating at 9.06 T and equipped with 5-mm inverse probe with a z-gradient. All monodimentional spectra were acquired at 298 k with a spectral width of 8223.685 Hz. The spectra were processed using the Topspin software by applying the exponential function for resolution enhancement with a line broadening of 0.125 Hz before Fourier transformation. Spectra were aligned on the residual solvent signal at 2.50 ppm. The NMR spectra were reduced to integrated regions (buckets) of equal width of 0.04 ppm each in the range of 0.40-10.50 ppm, excluding solvent and water regions from 2.47-2.52 ppm and from 3.31to 3.34, respectively. Buckets were scaled with respect to the total spectrum intensity, thus taking into account the different composition of samples. The metabolites ppm were verified from in house library [15, 16, 17,].

Statistical analysis

The data of table 1 were means of three separate experiments and the variations were detected using analyses of SPSS 17.0. The FTIR and ^{1}HNMR instrumental analyses were three times repeated. Significance of spectral numbers was calculated at ($P<0.05$).

RESULTS

pH prior and after plants incubation

The acidic water starting pH was (5.0), which was changed after drugs addition. The lowest pH was 5.25 and detected in the hypersensitive solution, followed by the painkiller solution; pH 5.59, vitamins; pH 5.82, and finally the pH 7.7 was measured in both calcium and the mixture solutions. When the plants were drugs-incubated for 30 minutes, the former pHs had exhibited an increase towards neutral and slight alkaline media (pHs: 7.01-7.84, Table 1).

Characterization of FTIR spectra of P. *sativum* leaves

The FTIR spectra viewed five regions. The first region was a major peak appeared within the range from 4000 till 2800cm^{-1}, and was corresponding to both-OH and- CH-symmetrical and asymmetrical stretching bands of the cell membrane structural fatty acids. About +8 cm-1 shift was detected in leaves incubated with calcium, and +16 cm^{-1}shift was detected when calcium was replaced with the hypersensitive, painkiller, and drugs mixture (Table 2). The second region: 1700-1500 cm^{-1} is assigned to amide I and amide II of proteins. Both peaks of this region were found at

Figure 1 : Variations of water pH after mixed with drugs and after incubation with *P. sativum* and *D. carota* for 90 minutes at 37 °C. The starting pH of water was 5.0.± is the standard deviation of the mean±SD. * significance of values compared to 'control'.

drug	pH prior incubation period	pH after incubation period
Non	5.0±0.03	7.01±0.0.05
Calcium	7.7±0.05*	7.58±0.01*
Vitamins	5.82±0.02	7.44±0.02*
Painkiller	5.59±0.1*	7.29±0.05
Hypersensitive	5.25±0.05	7.41±0.1
Mixture	7.7±0.02*	7.84±0.05*

Table 2. The FTIR spectra of drugs-incubated *P. sativum* leaves and *D.carota* roots in water (pH 5.0). Figures represented wave number (cm^{-1}) of bands in each region. "*"significant shifts compared to control "non".

plant	drug	Region I I I2800	Region II 1700-1500	Region III 1500-1200	Region IV 1200-900	RegionV 900 I
P. sativum	non	3440.8	1651.0 1519.8	1458.1 1396.4	1095.5	-
	calcium	3448.5	1651.0 1519.8	1458.1 1396.4	1095.5	-
	vitamins	3440.8	1651.0, 1519.8	1458.1 1396.4	1080.1*	570.9* 524.6* 470.5*
	painkiller	3456.2*	1651.0, 1519.8	1458.1 1396.4	1087.8*	570.9* 524.6* 470.6* 455.2*
	hypersensitive	3456.2*	1651.0, 1519.8	1396.8*	1090.1	524.6* 470.8*
	mixture	3456.2*	1651.0, 1519.8	1396.4*	1087.8	645.5*
D. carota	non	3425.3	1651.0	1396.4 1334.5 1249.9	1064.6	632.6 416.6
	calcium	3440.8	1651.0	1396.4 1342.4* 1249.8	1072.3	617.2* 586.3* 524.6*
	vitamins	3425.3	1651.0 1558.4*	1458.1* 1373.2* 1342.4* 1249.8	1056.8	594* 524.6* 455.2*
	painkiller	3440.8	1651.0 1542.9*	1396.4	1080.1*	570.9*
	hypersensitive	3409.9*	1620.1*	1404.1 1334.6 1249.8	1064.5	586.3* 524.6*
	mixture	3417.6	1635.5*	1404.1 1334.6 1257.5	1064.6	586.3* 524.6*

1651.0 and 1519.8 cm^{-1}, and were dominant in the spectra produced from differential incubational conditions. The third region: 1500-1200 cm-1 is amide III signature of proteins. In this region, the two peaks which have been appeared under normal conditions were located at 1458.1, 1396.4 cm^{-1}, and were not shifted under drugs incubation except 1458.1 cm^{-1} which has disappeared when P. *sativum* leaves were incubated with the hypersensitive and the mixture. The fourth region 1200-900 cm-1 has one peak only appeared at 1095.5 cm^{-1}. That peak was shifted under incubations with vitamins, painkiller and the mixture (Table 2). The fifth region are less than 900 cm-1peaks corresponding to OCH_3-polysaccharides. Large variations and

Table 3: Variations in the transmittance intensities of *P.sativum* and *D. carota* plants incubated with drugs in water "pH 5.0" for 90 minutes at 37 °C. The intensities were compared to intensities of drugs-free plants (non). "*"significant shifts.

plant	drug	Region I 2800	Region II 1700-1500	Region III 1500-1200	Region IV 1200-900	Region V 900
P. sativum	non	24	56	64	75	-
	calcium	26	60	68	80	-
	vitamins	4*	38*	54*	70	74*
	painkiller	2*	27*	47*	62*	74*
	hypersensitive	4*	32*	52*	68*	74*
	mixture	2*	34*	47*	62*	80*
D. carota	non	1	22	38-54	22	54
	calcium	1	20	32-54	34*	58
	vitamins	22*	45*	65*	46*	70*
	painkiller	4	40*	54	60*	70*
	hypersensitive	6	28	27*	26	48*
	mixture	4	25	25*	18	58

shifts were determined under this region and the whole region was barely detectable in the control or when plants were incubated with calcium incubation (Table 2).

The predominant variations of transmittance intensities in all drug incubated plants were not significantly observed in calcium-plants (Table 3).

Characterization of FTIR spectra in root of D. *carota*

Spectral analyses of roots showed deviations within the first region peak when they were incubated with drugs except vitamins (Table 2). In the first region, +15 cm^{-1}shift was found when roots were incubated with calcium and painkiller. Moreover, -16 and -8 cm^{-1} were shifted after roots exposure to hypersensitive and the mixture, respectively. Regarding the second region, new bands and shifts appeared with vitamins and painkiller incubations (data not shown). With respect to third region located at region: 1500-1200 cm^{-1}, the control peaks stabilized at 1334.5, 1249.9 cm^{-1} had disappeared under painkiller treatment. Added to 1458.1 cm^{-1} appearance and 1396.4 cm^{-1} disappearance in roots spectra exposed to vitamins. In the fourth region, the highest up- and down-shifts were recorded in the calcium, vitamins and the painkiller exposures. Multiple up and down fluctuations have been occurred similarly within the fifth region under different drugs incubations (Table 2).

Transmittance intensity of peaks of drugs-incubated pants had changed adversely compared to control plants. Calcium was the consistent with the control, whereas the vitamins and the painkiller were not (Table 3).

^{1}HNMR spectroscopy

Data of ^{1}HNMR spectra were available for total 43 metabolites (Table 4). Precise analyses had confirmed multiple shifts in fingerprinting of $CDCl_3$ extracts of 30 minutes incubated plants with drugs at 37° C. Exposed P. *sativum* leaves to the hypersensitive drug exhibited nine disappeared metabolites, were: lipids, steroids, GABA, chlorogenic, triglycerides, malic acid, β-glucose, α-glucose, and cinnamic acid. In addition to ten newly released metabolites: citric acid, inositol, alcohol, serine, kaempferol, apigenin, fumaric, caffeic, chlorogenic and ferulic. When the leaves were exposed to the painkiller, the seven metabolites which had disappeared were: lipid, chlorogenic, linoleic, β-glucose, α-glucose, triglycerides and apiin (Table 4). In parallel to the six new metabolites: esters, raffinose, diglycerides, sucrose, cinnamic acid and kaempferol.

The ^{1}HNMR spectra of *D. carota* exposed to the hypersensitive drug have detected seven metabolic disappearances, were: lipid, malic acid, aldehyde, malic acid, triglycerides, apiin and cinamic acid. However, the serine has solely appeared under that treatment. When *D. carota* was incubated in the painkiller, eleven disappearances of metabolites were resulted: lipid, threonine, malic acid, aldehyde, linoleic acid, asparagine, raffinose, malic acid, α-glucose, apiin and cinnamic acid. Added to six metabolites which have been appeared under the same incubation, and were: steroids, isoleucine, rhamnose in flavonoids, citric acid, serine and the diglycerides (Table 4).

DISCUSSIONS

In the literature, drugs can alter the pH of the stomach converting it to neutral[18] which was in consistence with the present results, as drugs were found to raise the acidic water pH towards the neutral, particularly in the presence of the plants (Table 1).

Table 4: ^{1}HNMR spectra of $CDCl_3$ extracts showing metabolic fingerprinting of *P. sativum* leaves and *D. carota* roots incubated with hypersensitive and painkiller drugs. Red fields illustrated disappearances of metabolites and green shades illustrate the appeared metabolites compared to unincubated plants "non". (Brahmi et al., 2015; Szajdak et al., 2015)

			P. sativum			*D.carota*		
No	**ppm**	**metabolites**	**non**	**hypersensitive**	**painkiller**	**non**	**hypersensitive**	**painkiler**
1	0.7	Lipid terminal methyl	X		X	X		
2	0.78	steroids	X		X			X
3	0.89	fatty acids	X	X	X	X	X	X
4	0.97	maRubenol	X	X	X			
5	0.99	valine	X	X	X	X	X	X
6	1.01	isoleucine	X	X	X			X
7	1.1	Rhamnose in flavonoid	X	X	X			X
8	1.26	fatty acids	X	X	X	X	X	X
9	1.32	threonine	X	X	X	X	X	
10	1.48	alanine	X	X	X	X	X	X
11	1.5	Lipid	X	X		X	X	X
12	1.84	GABA	X		X			
13	2.1	Chlorogenic	X			X	X	X
14	2.15	Glutamine	X	X	X	X	X	X
15	2.36	fatty acids	X	X	X			
16	2.36	glutamic acids	X	X	X			
17	2.39	Malic acid				X		
18	2.43	aldehydes				X		
19	2.72	citric acid		X				X
20	2.78	linoleic acid	X	X		X	X	
21	2.88	asparagine	X	X	X	X	X	
22	3.6	Inositol		X				
23	3.65	alcohols		X				
24	3.75	Serine		X			X	X
25	4.05	esters,			X			
26	4.15	triglycerides	X		X			
27	4.2	Raffinose			X	X	X	

28	4.22	diglycerides				X					
29	4.3	Malic acid	X			X		X			
30	4.6	beta-glucose	X								
31	5.03	diglycerides									X
32	5.24	alpha-glucose	X					X	X		
33	5.28	triglycerides	X	X				X			X
34	5.36	unsaturated fatty acids, esters, oleic acid	X	X		X		X	X		X
35	5.4	sucrose				X		X	X		X
36	5.42	apiin	X	X				X			
37	6.3	cinnamic acid	X			X		X			
38	6.35	kaempferol		X		X					
39	6.47	apigenin-7-derivatives		X							
40	6.56	fumaric acid		X							
41	7.03	caffeic acid		X							
42	7.07	chlorogenic acid		X							
43	7.1	ferulic acid		X							

The spectra of FTIR and NMR were reading the metabolic fingerprinting scenario consistently

Fatty acids are important in forming the cellular membrane bilayer and used as signals[19]. The structure of fatty acids and lipids has been manipulated when plants were incubated with the hypersensitive and painkiller drugs. Which was observed as shifts in the first FTIR region spectra. The ^{1}HNMR spectra supported the previous result recording disappearance of lipids, steroids, and triglycerides and the appearance of diglycerides (Table 4). Here, we hypothesized variations of lipid biosynthetic pathway from fatty acids and glycerolipid [20].

The FTIR spectra were reading the status of protein amides I, II, III at both the second and the third regions. For example, P. *sativum* exposure to calcium, vitamins and the painkiller resulted in unchanged spectral positions, and the ^{1}HNMR of the same plant in the painkiller were not changed regarding their amino acids profile. From table 4, it was shown that the amino acids: valine, alanine, isoleucine, threonine and asparagine were detected in the control and in the painkiller. Furthermore, seine was absent both in control and painkiller in P. *sativum* (Table 4). The ^{1}HNMR spectra of *D. carota* roots incubated in painkiller had viewed an induction of both serine and isoleucine, a result confirmed by FTIR spectra declaring a new band located at 1542.9 cm^{-1} (Table 2). Moreover, asparagine and threonine disappeared spectra upon painkiller treatment were in coordination with FTIR spectra which recorded many disappeared amides bands compared to drug-free roots (Tables 2&4).

Serine sudden appearance in ^{1}HNMR spectra in hypersensitive treatment in both plants can be explained as altered protein wavenumber and intensity on their FTIR regions (Tables 2&3). Protein status confirmed that the amino acids biosynthetic pathway must has been manipulated[21], likely due to the disappearance of glucose as detected from the ^{1}HNMR spectra, as glucose is known as indirect precursor of some amino acids via the pyruvate[22]. That was confirmed by the predominant disappearance of α-glucose, β-glucose, raffinose and sucrose ^{1}HNMR spectra in both plants (Table 4). The later could be also explaining the alterations of carbohydrates and polysaccharides spectra at the fourth and fifth regions of FTIR spectra. Furthermore, GABA protein disappearance from ^{1}HNMR spectra of P. *sativum* after hypersensitive treatment could explain many physiological and metabolic fingerprinting alterations particularly that GABA ruled in ion channels and ion transport (Table 4).

The predominant appearance of chlorogenic, caffeic, kaempferol, ferulic, apigenin and fumaric in P. *sativum* incubated in hypersensitive drug is pointing to the induction of fumaric and shikimic acids pathways under the drug.

CONCLUSION

The current results had visualized that drugs-plants interaction in stomach like conditions had altered plants lipid,

steroids and fatty acid and subsequently had influenced lipid biosynthetic pathway and cellular membranes structure. Drugs influenced amides and protein structure and pathways as appeared from the shifted FTIR and ^{1}HNMR spectra. The carbohydrates were also affected from shown glucose, raffinose and sucrose shifted spectra. The effect of painkiller and hypersensitive were the worst among all studied drugs. Considering the low doses of used drugs for plants incubation -as each tablet was divided into 24 parts, and one part only was dissolved in 250 ml acidic water, pH5- these results are alerting for more serious shifts in plant's metabolic fingerprinting upon eating a full tablet in a faded stomach with such plants. The chemical shifts hints to possible originated complexes from drugs interactions with the primary and secondary metabolites in these plants, which could also suggest a solution for the conflict regarding the reasons of inappropriate drugs absorption or actions in the presence of food or vegetables inside the stomach.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Avoid food-drug interactions. A guide from the National Conssumers League and U.S. food and drug administration, U.S. Department of Health and Human Services). 2013. www.nclnet.org or www.fda.gov/drugs.

2. Medication Interactions: Food, Supplements and Other Drugs. American Heart Association, 2014.

3. The National CML Society. Drugs and Food Interactions. 2014.

4. Pappas CS, Tarantilis PA, Harizanis PC. App Spec. 2003: 57:23-27.

5. Mularczyk-Oliwa M, Bombalaska A, Kaliszewski M, Wlodaski M, Copczynki K, Kwasny M, Szpakowska M, Trafny EA. Comparison of fluorescencespectroscopy and FTIR in differentiation of plant pollens. Spec Acta Part A: Mol Biom Spec. 2012: 97: 246-254.

6. Song SY, Lee YK, Kim IJ. Sugar and acid content of Citrus prediction modeling using FT-IT fingerprinting in combination with multivariate statistical analysis. Food Chem. 2016: 19:1027-1032.

7. Skotti E, Kountouri S, Bouchagier P, Tsitsigiannis DI, Polissiou M, Tarantilis PE. FTIR spectroscopic evaluation of changes in the cellular biochemical composition of the phytopathogenic fungus Alternaria alternate induced by extracts of some Greek medicinal and aromatic plants. Spec Acta Mol Biom Spec. 2014: 127:463-472.

8. Mingolet A, Derenne E, Smolina M, Wood BR, Goormaghtigh E. FTIR spectral signature of anticancer drugs. Can drug mode of action be identified? Biochim Biophy Acta. 2016: 1864:85-101.

9. Holmes E, Tang H, Wang Y, Seger C. The assessment of plant metabolite profile by NMR-based methodologies. Planta Med. 2006: 72: 771-785.

10. Kim HK, Choi YH, Verpoorte R. NMR-based metabolomics analysis of plants. Nat Prot. 20105: 536-549.

11. Ulrich-Merzenich G, Zeitler H, Jobst D, Pankek D, Vetter H, Wagner H. Application of the-omic-technologies in phytomedicine. Phytomedicine. 2007: 14:70-82.

12. Wang M, Lambers RJAN, Korthout, HAA, Van Nesselrooij JHJ, Witkamp RF, Van der Heijden R, Voshol PJ, Havekes LM, Verpoorte R, Van der greef. Metabolomics in the context of systems biology: bridging traditional Chinese medicine and molecular pharmacology. Phy Res. 2005: 19:173-182.

13. Yilmaz A, Nyberg NT, Molgaard P, Asili J, Jaszewski JW. 1H NMR metabolic fingerprinting of saffron extracts. Metabolomics. 2010: 6:511-517.

14. Petrakis EA, Cagliani LR, Polissoiu MG, Conssoni R. Evaluation of saffron (*Crocus sativus* L.) adulteration with plant adulterants by 1H NMR metabolic fingerprinting. Food Chem. 2014: 173:890-896.

15. Szajdak LW, Maryganove V, Skakoviskii E, Tychinskaya L. 1H and 13C NMR spectroscopic studies of hexane-extractable lipids from soil under shelterbelts of different age and composition of plants. Chemosphere. 2015: 119:1422-1427.

16. Brahmi N, Scognamiglio M, Pacifico S, Mekhoukhe A, Madani K, Fiorentino A, Monaco P. ^{1}HNMR based metabolic profiling of eleven Algerian aromatic plants and evaluation of their antioxidant and cytotoxic properties. Food Res Int. 2015: 76:334-341.

17. Gogna N, Hamid N, Dorai K. Metabolomic profiling of the phytomedicinal constituents of Carica papaya L. leaves and seeds by ^{1}HNMR spectroscopy and multivariate statistical analysis. J Pharm Biomed Anal. 2015: 115:74-85.

18. Linda B, Lentz A, Turner RE. Food/drug and drug/nutrient interactions: what you should know about your medications. University of Florida EFAS Extension. 2009.

19. Harwood JL. Recent advances in the biosynthesis of plant fatty acids. Biochem Biophy Acta. 1996: 1301:756.

20. Beisson LY, ShoRosh B, Beisson F, Andersson MX, Arondel V, Bates PD, Baud S, Bird D, Debono A, DuRette TP, Franke RB, Graham IA, Katayama K, Kelly AA, Larson T, Markham JE, Miquel M, Molina I, Nishida I, Rowand O, Samuels L, Schmid KM, Wada H, Welti R, Xu C, Zallot R, Ohlrogge J (2010) New insights on the organization and regulation of the fatty acid biosynthetic network in the model higher plant Arabidopsis thaliana. Acyl-lipid metabolism Arabidopsis Book 8:165.

21. Gaufichon L, Reisdoref-Cren M, Rothstein SJ, Chardon F, Suzuki A. Biological functions of asparagine synthetase in plants. Plant Sci. 2010: 179:141-153.

22. Yang J, Sung-Ryul Kim SR, Lee SK, Choi H, Jeon JSG, An G (2015) Alanine aminotransferase 1 (OsAlaAT1) plays an essential role in the regulation of starch storage in rice endosperm. Plant Sci. 240:7989.