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Green Extraction: Evaluation of Lipid Contents of Chicory (*Cichorium Intybus*) Seeds Extracted By Four Different Extraction Methods Using GC/MS and HPTLC.

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ABSTRACT

Fixed oils (lipids) of Chicory (*Cichorium intybus* L.) plant seeds was extracted by four different extraction methods viz. percolation, microwave assisted extraction (MAE), ultrasonic assisted extraction (UAE) and supercritical fluid extraction (SFE). The total yield of extracted lipids of the studied extraction methods were 10.2 g, 15.5 g, 17.64 g. and 12.90 g respectively. Linoleic acid (C18:2), the characteristic fatty acid of Chicory, was evaluated using GC/MS. The results revealed that both MAE, UAE and SFE enhanced the extraction efficiency of the fatty acid of Chicory where MAE gave the highest percentage yield (72.86%), whereas (UAE) gave (67.08%) and (66.5%) for SFE compared to the percolation method (61.85%) respectively.Also, the obtained data revealed that the total lipids (mono, digly. (41.66%, 28.72%, 32.78%, 40.13%) & triglyc. (37.14%, 55.88%, 48.72%, 36.54%), total free fatty acids (13.52%, 15.19%, 14.56%, 12.64%) and total fatty acids methyl esters (fames) (2.78%, 0.38%, 0.69%, 1.82%) of the four different extraction methods of Chicory *(Cichorium intybus* L.*)* was evaluated using High Performance Thin Layer Chromatography (HPTLC).Microwave-assisted extraction (MAE), ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE) not only enhanced the total lipid extraction but also saved time, reduced the solvents use and produced, ecologically, green technologies.

Keywords: *Cichorium intybus*, linoleic acid, HPTLC, GC/MS, microwave-assisted extraction, ultrasonic-assisted extraction, supercritical fluid extraction, green technology.

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INTRODUCTION

Chicory (*Cichorium intybus* L.) belonging to family Asteraceae (Compositae) has a history of medicinal use especially of great value for its tonic effects upon the liver and digestive tract. Chicory was also widely used to treat diabetes mellitus. The seeds of *Cichorium intybus* L. showed to have high levels of nutritionally important components and may be of significant importance in the formulation of diets for human and animals [1]. The essential fatty acid, linoleic acid was the predominant fatty acid accounted for over 76 % of the total fatty acids in the seeds, with lower saturated/unsaturated ratios (about 0.11) making the plant potentially a superior source of nutritional oil [2].

Extraction forms the first basic step in medicinal plant research because the preparation of crude extracts from plants is the starting point for the isolation and purification of chemical constituents present in plants. Yet the extraction step remains often a neglected area, which over the years has received much less attention and research [3,4].

The traditional techniques of solvent extraction of plant materials are mostly based on the correct choice of solvents and the use of heat and/or agitation to increase the solubility of the desired compounds and improve the mass transfer. Usually the traditional technique requires longer extraction time thus running a severe risk of thermal degradation for most of the phyto-constituents. Thus, the major significant shortcomings of traditional extraction techniques is the lengthy extraction time that can be 8, 16, and 24 hours or more, which results in consumption of considerable time and heat energy [5,6]. The fact that one single plant can contain several secondary metabolites makes the need for the development of high performance and rapid extraction methods an absolute necessity [7]. Keeping in pace with such requirements, recent times has witnessed the use and growth of new extraction techniques with shortened extraction time, reduced solvent consumption, increased pollution prevention concern and with special care for thermo labile constituents. Novel extraction methods including microwave assisted extraction (MAE) [8], supercritical fluid extraction (SCFE) [9-11], accelerated solvent extraction (ASE) [12], Subcritical Water Extraction (SWE) [13] and ultrasound extraction (USE) [14], have drawn significant research attention in the last decade [15]. If these techniques are explored scientifically, they can provide an efficient extraction technology for ensuring the quality of herbal medicines worldwide.

The main aim of this work is to do a comparative study to evaluate the use of MAE, USE and SFE in fixed oil extraction from chicory seeds and compare the extraction yield and fatty acids profile of the oil with solvent extraction method (percolation).

MATERIALS AND METHODS

Plant Materials

Chicory (*Cichorium intybus* L.) seeds were purchased from the local market from "Giza Company for Seeds and Herbs". The company depends mainly on exportation of raw plant materials to USA and Europe. This means that the standard of the materials quality is high. The plant seeds were authenticated by Professor Kamal Zayed, Botany Department, Cairo University. A voucher specimen was kept in the herbarium of National Research Center (NRC) of Egypt.

Methods of Extraction

Fixed oils (lipids) of Chicory (*Cichorium intybus*) plant seeds was extracted by four different methods of extraction for *viz.* Percolation, Microwave-Assisted Extraction (MAE), Ultrasonic-Assisted Extraction (UAE) and Supercritical Fluid Extraction (SFECO2) as follows:

Conventional Extraction Method

100 g of powdered Chicory seeds were percolated with chloroform/methanol (2:1) (2000 ml) and recycling for 4 days. After complete exhaustion, the chloroform/methanol extract was evaporated under vacuum at 40^{o}C [16].

Microwave-Assisted Extraction (MAE)

100g of homogenous dried powdered seeds was mixed with 800 mL of chloroform/methanol (2:1), using opened system microwave apparatus, Mars CEM 240/50, model number 907511, serial number MD 3728. After allowing a preleaching time of 5 min, the suspension was irradiated with microwave at (about 60°C) for 20 min at microwave power 600 W. After extraction, the samples were centrifuged at 4000 rpm and the supernatant evaporated under reduced pressure at 40°C [17].

Ultrasonic-Assisted Extraction

100g of homogenous dried powdered seeds was mixed with 800 mL of chloroform/methanol (2:1) for 20 min at power 400 W (amplitude 0.5 and rotation 70 cycles) using an Ultrasonic Processor UP400S (400 watts, 24kHz, Hielscher) direct sonication, ultrasonic probe with a tip diameter of 20 mm, fitted into the flask and the tip was inserted at the half height of the extraction solvent. After extraction, the extract was centrifuged at 4000 rpm and the supernatant evaporated under reduced pressure [18].

Supercritical Fluid Extraction

An Applied Separation system in the SFE mode was used for all the extractions. The extraction vessel was a 10 mL stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 200 bar and temperatures of 50 ºC for a duration of 15 min, in static mode, followed by 3 hrs, in dynamic mode. Flow rate of CO_2 gas 1L/min [19].

Sample preparation for analysis

0.5 gm of each sample extract was taken in 10 ml chloroform and 2 gm anhydrous sodium sulphate was added, vortexed.

Sample preparation for GC/MS

One ml of each chloroform extract was taken, evaporated till dryness with argon gas. Then, to each sample 2 ml of 1.5% H_2SO_4 in methanol was added and left for 3 hours in a heating box at 90°C. The hydrolysis reaction was stopped by the addition of 2 ml water to each tube. The fatty acids were then extracted with shaking the aqueous layer with hexane, passing the hexane layer over anhydrous sodium sulfate to remove water traces and concentrating it at 40°C till 100µl. The concentrated hexane layers were analyzed by GC/MS.

GC/MS apparatus and conditions

GC/MS was carried out using an HP5890 Series II Gas Chromatography, HP 5972 Mass Selective Detector and Agilent 6890 Series Auto sampler. A Supelco MDN-5S 30 m by 0.25mm capillary column with a 0.5 µm film thickness was used with helium as the carrier gas at a flow rate of 1.0 ml/min. The GC oven temperature was programmed at an initial temperature of 130˚C for 1 minute, then heated up to 300˚C at 5˚C/min and held at 300˚C for 5 minutes. Injector and detector temperatures were set at 250˚C. Mass spectrometry was run in the electron impact (EI) at 70eV. The identification of the chemical constituents were determined by their GC retention times, interpretation of their mass spectra and confirmed by mass spectral library search using the National Institute of Standards and Technology (NIST) database.

Sample preparation for HPTLC

100 µL of each chloroform extract was taken and diluted to one ml, then subjected to HPTLC under the following conditions:

Stationary phase

20 x 10 cm glass plates HPTLC silica gel 60 F_{254} (Merck).

Sample application

Apply 5 µL of each tested sample as 6 mm band, 2 mm apart, 8 mm from the lower edge and 15 mm from left and right edges of the plate.

Temperature and humidity

Record temperature and humidity in the lab. If the relative humidity exceeds 50% RH, condition the plate to about 30% RH using a suitable device.

Chromatography

Developing solvent:

pet.ether/ether/formic acid (90/10/2).

Chamber

Pour 12 ml of developing solvent in the right trough of chamber and 25 ml in the left one. Allow the chamber to saturate for 20 min.

Development

Migration distance of developing solvent on the plate is 85 mm from lower edge of the plate.

Drying

Dry the plate for 10 min.

Preparation of Derivatizing Reagents

Copper Sulphate Reagent

20 gm of Copper Sulphate Pentahydrate + 200 ml Methanol (at less than 20C°). Then, under cooling with ice, add 8 ml of Sulfuric Acid (98%) and 8 ml Ortho-Phosphoric Acid (85%).

Derivatization with Copper Sulphate Reagent

Dip the plate into the tank of the immersion device charged with 200 ml of Copper Sulphate Reagent, by placing the plate in holder of immersion device (speed: 5, time: 5sec.), allow the plate to dry for 1 min inside the hood and heat in oven for 30 min at 140 C°.

- *CAMAG* Automatic TLC Scanner with CATS evaluation Software.
- Wave length scan at 420 nm.
- Scanning speed 20 mm/s.

Standard Solution Preparation

Each standard solution was diluted to concentration of 5mg in one ml $CHCl_3$ and 5µl was injected.

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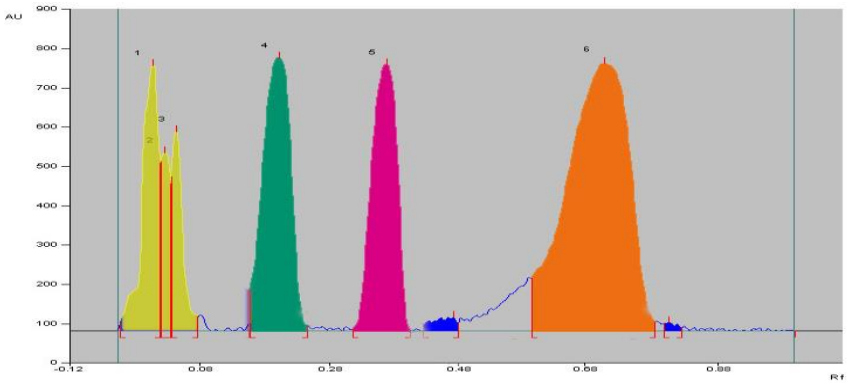


Peaks 1,2,3 represent mono.. & diglycerides R_f (-0.04 to 0.08).
Peak 4 represents free fatty acids R_f (0.15 to 0.24).
peak 5 represents tri-glycerides R_f (0.32 to 0.41) respectively.
Peak 6 represents fatty acid methyl esters R_f (0.57 to 0.76)

RESULTS

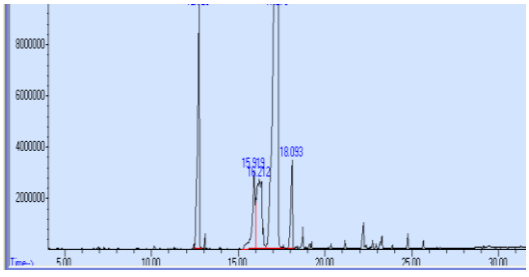
Effect of Extraction Method on the Total Lipids:

Table 1: Showed the total extract yields/100 g:

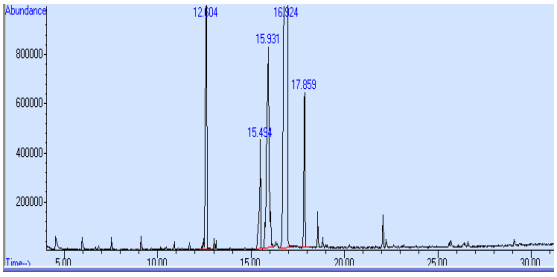
	Perculation	Microwave	Sonicator	CO2
Shiquria	10.2 g	15.5 g	17.64 g	12.90

The goal of this work is to compare classical (traditional) extraction techniques of plants with unconventional methods from Chicory (*Cichorium intybus*) Seeds plant with respect to amount of extracted material and chemical composition of the extracts. Table 1 shows the mass yield (g of extract/100 g of sample) obtained by four different extraction techniques in the best conditions. The results of different extraction methods employed being presented. The total oil yield percent of Chicory (*Cichorium intybus*) plant seeds, obtained by percolation method, was 10.2 g, showing the highest oil content for 4 days. Whereas, that obtained by the new methods (15.5 g, 17.64 g. and 12.90g for MAE, UAE and SFE respectively, within 20 min, 20 min and 3 hours extraction time, for once) showed that these techniques, can improve the extraction yield at shorter reaction times and at low or moderate costs. To some extent, SFE showed to be inconvenient technique for extraction of lipids, depending on yield comparison.

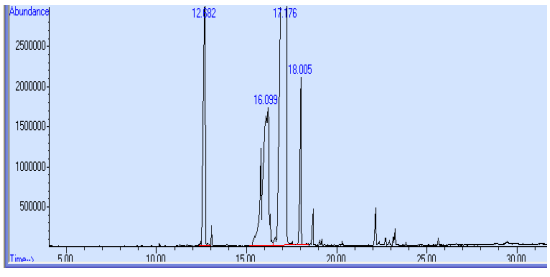
The Fatty Acid Composition of *Cichorium intybus* L.:



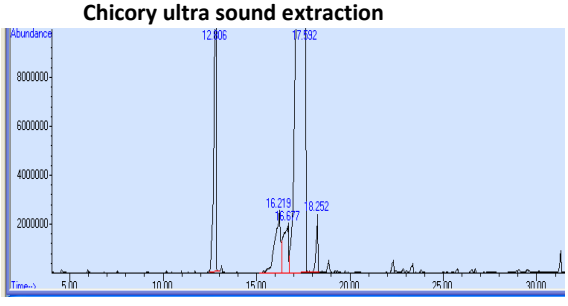
Chicory percolation extraction



Chicory ultra sound extraction



Chicory microwave extraction



Chicory CO_2 extraction

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Table 2: Comparative Study of Fatty Acid Methyl Ester of Different Extraction Techniques of Chicory (*Cichorium intybus*) plant seeds

Retention times	12.73	15.92	16.21	17.27	18.09
Fatty Acids %	Palmitic acid (C16-0)	Stearic acid (C18-0)	Oleic acid (C18-1)	Linoleic acid (C18-2)	Linolenic acid (C18-3)
Percolation	14.28	6.65	12.42	61.85	4.80
Sonication	12.38	3.74	13.20	67.08	3.60
Microwave	9.55	-	14.30	72.86	3.29
CO2	13	-	12.56	66.5	7.94

Linoleic acid (C18:2) is a characteristic fatty acid of the plant. The fatty acid composition of *Cichorium intybus* L**.** seeds is mainly linoleic acid, palmitic acid, and few amount of the essential fatty acid linolenic acid.

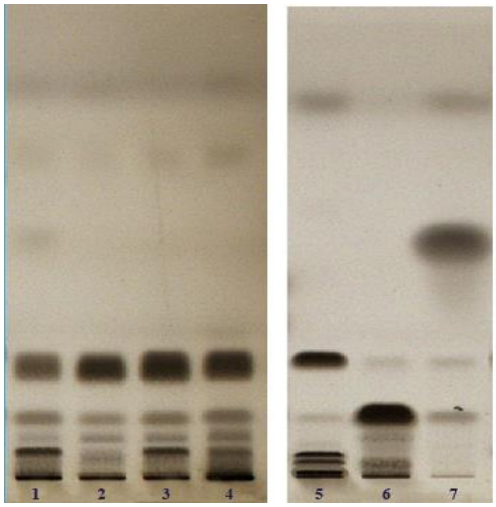
In our study, the linoleic acid is highly increased by using the new extraction methods. Linoleic acid is an important fatty acid, this is especially true for the growth and development of infants. Linoleic acid is a polyunsaturated fatty acid that is part of the Omega 6 fatty acids family. It is a precursor for a hormone like substance prostaglandins. Prostaglandins are substances found in the body's cells. Linoleic acid is an essential fatty acid which the body cannot produce itself so it must be included in the diet.

In our study GC/MS has shown that linoleic acid is the major fatty acid. MAE, UAE and SFE gave very similar profile as shown by lipids obtained from percolation.

The following fatty acids were detected in *Cichorium intybus* L**.** seeds: C16:0, C18:0, C18:1, C18:2 and C18:3. Reliability and accuracy of the analytical methods for the detection of fatty acids were ensured by the use of the certified reference matrix that consisted of a mixture of 37 FAME standards triglyceride standard, free F.A. standard and glycerol standard. The contents of the particular fatty acids are expressed as percentages of the sum of all of the fatty acids analyzed.

Comparative Study of Chicory Extraction Methods

Documentation of Derivatized Plate (Copper Sulphate Reagent):



Derivatized Plate @ White R

Track 1: Chicory extracted by percolation
Track 2: Chicory extracted by microwave
Track 3: Chicory extracted by sonicator
Track 4: Chicory extracted by CO2
Track 5: Standard Glycerides (mono …, di…, Tri-glycrides)
Track 6: Standard Free Fatty Acids
Track 7: Standard Fatty Acid Methyl Esters (fames)

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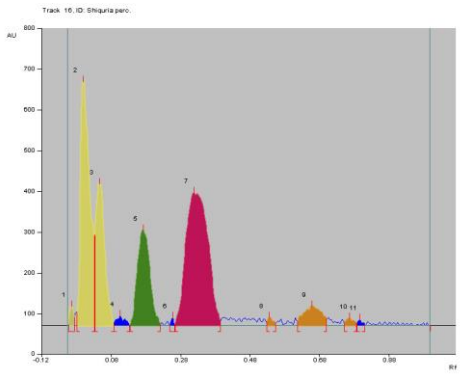
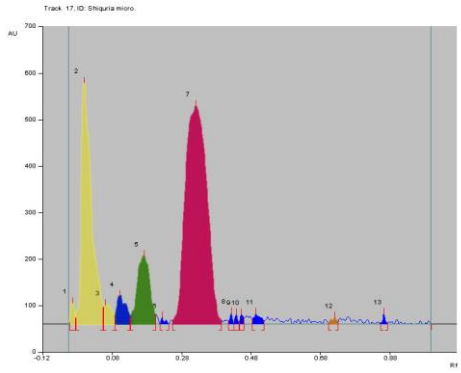
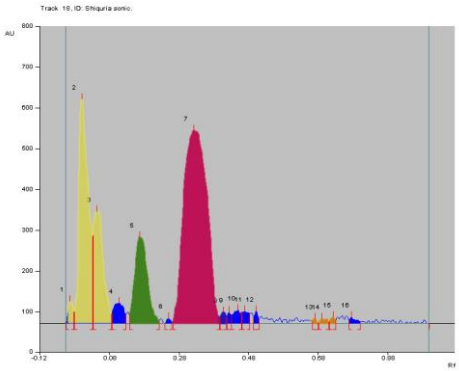


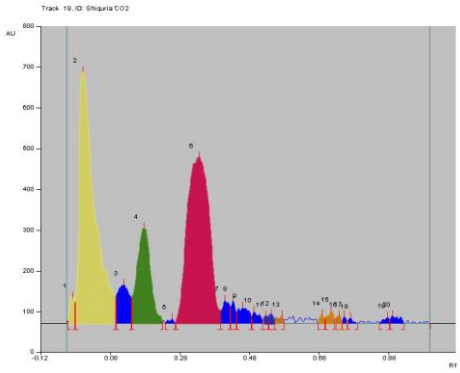
Percolation Extraction Method



Microwave Extraction Method



Sonicator Extraction Method



CO2 Extraction Method

Table 3: The Total Lipids of Different Extraction Techniques of Chicory (*Cichorium intybus*) plant seeds

Class of comp	R_f Range	Extraction Method%			
		Percolation	Microwave	Sonicator	CO2
Mono. & Diglyc.	(-0.04 to 0.08)	41.66%	28.72%	32.78%	40.13%
Triglyc.	(0.32 to 0.41)	37.14%	55.88%	48.72%	36.54%
Total Free F. A.	(0.15 to 0.24)	13.52%	15.19%	14.56%	12.64%
Total Fames	(0.57 to 0.76)	2.78%	0.38%	0.69%	1.82%

Data obtained by Table (3) revealed that the total lipids (Mono, Di (41.66%, 28.72%, 32.78%, 40.13%) & Triglyc. (37.14%, 55.88%, 48.72%, 36.54%), Total Free F. A. (13.52%, 15.19%, 14.56%, 12.64%) and Total Fames (2.78%, 0.38%, 0.69%, 1.82%) of the four different extraction methods of Chicory (*Cichorium intybus* L.) was evaluated using High Performance Thin Layer Chromatography (HPTLC).

Microwave - assisted extraction (MAE), Ultrasonic -assisted extraction (UAE) and supercritical fluid extraction (SFE) not only enhanced the total lipid extraction but also saved time, reduced the solvents use and produced, ecologically, green technologies.

CONCLUSION

The present study indicates that microwave assisted extraction and ultrasound assisted extraction can be used as a desirable alternative to conventional oil extraction techniques. The major advantage of these methods is the reduced time of extraction and energy consumption costs, when is compared to conventional methods. It allows also for better retention and availability of desirable nutraceuticals, such as triglycerides and free fatty acids (FFA) in the extracted oil where the percentage is very highly improved by these techniques. This can be a new step to produce nutritional vegetable oils with higher nutrition value.

The four extraction techniques were qualitatively the same, but the GC/MS analysis, HPTLC and the scanner showed quantitative difference.

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REFERENCES

[1] Roohi Zaman and Sadiya Noorul Basar. Research Journal of Pharmaceutical Sciences 2013;2(8): 1-4.
[2] Gu WenYing1, Li Jin Gui. J Animal Plant Sci 2012;13(2): 1736-1746
[3] Romanik G, Gilgenast E, Przyjazny A, Namiesnik J and M Kaminski. J Biochim Biophys Methods 2007 (Article in press).
[4] Smith RM. J Chromatogr A 2003;1000: 3-27.
[5] Luque de Castro MD, and Garcia-Ayuso LE. Anal Chim Acta 1998;369: 1-10.
[6] Pastot E Vazquez, R Ciscar and M De la Guardia. Anal Chim Acta 1997;344: 241-49.
[7] Nyiredy S. J Chromatogr B 2004;812: 35-51.
[8] Huie CW. Anal Bioanal Chem 2002;373: 23-30.
[9] Taylor LT. Supercritical Fluid Extraction, John Wiley & Sons, Inc., New York, NY., 1996.
[10] Jian Ya Ling, Guo Ying Zhang, Zhao Jie Cui, Chang Kai Zhang. J Chromatogr A 2007;1145:123–127.
[11] Sameh Baananou, Alessandra Piras, Bruno Marongiu, M. Assunta Dessì, Danilo Falconieri, Silvia Porcedda, Antonella Rosa, Naceur A Boughattas. African J Pharm Pharmacol 2012;6(10): 756-762.
[12] Luthria DL. Indian J Chem Sect B 2006;45:2291–2296.
[13] Miller DJ, & Hawthorne SB. J Chem Eng Data 2000;45:315-318.
[14] Luque-Garciá JL, & Luque de Castro, MD. Trends Anal Chem 2003;22:41−47.
[15] letellier M, Budzinski H, Charrier L. Capes S and Dorthe AM. J Anal Chem 1999;364: 228-37.
[16] Khaled A Shams, Nahla S Abdel-Azim, Wafaa A Tawfik, Heba D Hassanein, Mahmoud A Saleh and Faiza M Hammouda. J Chem Pharm Res 2015;7(4):1080-1088.
[17] Khaled A Shams, Nahla S Abdel-Azim, Sherin A Kamal, Husseiny A. Husseiny and Faiza M. Hammouda. J Chem Pharm Res 2015;7(4):725-728.
[18] Ibrahim A Saleh, Sherin A Kamal, Khaled A Shams, Nahla S Abdel-Azim, Elsayed A Aboutabl and Faiza M Hammouda. Res J Pharm Biol Chem Sci 2015;6(2):803-809.
[19] Faiza M Hammouda, Mahmoud A Saleh, Nahla S Abdel-Azim, Khaled A Shams, Shams I Ismail, Abdelaaty A Shahat and Ibrahim A Saleh. Afr J Tradit Complement Altern Med 2014;11(2):378-380