Journal of materials and Environmental Sciences
ISSN : 2028-2508

Copyright © 2017,

JMES, 2017 Volume 8, Issue 2, Page 551-556

http://www.jmaterenvironsci.com /

JMES

Essential oil of *Hertia cheirifolia* leaves : chemical composition, antibacterial and antioxidant activities

N. Segueni[1*], A. Zellagui[1,2], S. Boulechfar[2], K. Derouiche[1,2], S. Rhouati[1]

[1] *Laboratory of Natural Products and Organic Synthesis, Department of Chemistry, Faculty of Science, University Constantine 1, Algeria.*

[2] *Laboratory of Biomolecules and Plant Breeding, Life Science and Nature Department. Faculty of Exact Science and Life Science and Nature, University of Larbi Ben Mhidi, Oum El Bouaghi, Algeria*

Received 13 Nov 2016,
Revised 08 Dec 2016,
Accepted 13 Dec 2016

Keywords
- *Hertia cheirifolia*,
- Essential oil,
- GC-MS analysis,
- Antibacterial activity,
- Antioxidant activity

segueninarimane@yahoo.fr
Phone: +213794132791

Abstract

The essential oils obtained by hydrodistillation of *Hertia cheirifolia* leaves were analyzed by GC/MS analysis. Sixty two compounds were identified representing 78.29% of the total oil. The main constituents were found to be monoethylhexyl phthalate, (33.71%), valeranone, (6.90%), (-) Drimenin (6.71%) and benzene, tert-butyl (3.06%). The antibacterial activity of the leaf of *Hertia cheirifolia* was evaluated by disc diffusion method and tested against Gram-positive and Gram-negative bacteria. *Pseudomonas aeruginosa* ATCC27853 was the most resistant bacteria. The tested oil had moderate activity on *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Klebsiella pneumonia*. The essential oil was also screened for a possible *in vitro* antioxidant activity by DPPH free radical-scavenging test. The findings showed that percentage of reduction is 88.47 % at 1M.

1. Introduction

The genus *Hertia* with its twelve species is distributed over south and north Africa and southwest Asia [1]. *Hertia cheirifolia* (L) O.K also known as *Othonnopsis cheirifolia* Jaub. et Spach. is a small plant with yellow flowers, which grows in the border fields in the eastern part of Algeria and Tunisia [2, 3]. Lot of researches were performed concerning chemical composition and biological activities of Asteraceae but just few studies were focused on this genus. Previous phytochemical investigations on the genus *Hertia* reported the presence of eremophilenolides sesquiterpenes [4] and bakkenolide type sesquiterpenes. Steroids were also reported from chloroform extract [5]. In the same study various extracts of *H. cheirifolia* were also tested for their spasmolytic and anti-inflammatory activities. Tests were based on the contractile response effect on rat isolate smooth muscle and on the dose-related carrageenan induced paw oedema in rats. The antispasmodic effect of bakkenolide was found in the same range as that of alverine, a standard muscoltropic spasmolytic agent [5]. *H. cheirifolia* essential oil was investigated against two spotted spiders mite (*Tertanychus urticae*). Mite mortality increased with oil concentration and a reduction in fecundity was also observed [6].

Plant extracts are well used in herbal and pharmaceutical medicines. They are used also as spices and found numerous uses in cosmetics and perfumes [7]. They are considered as an interesting material for both medicinal and food industries due to their low toxicity and easy accessibility. Among plant extracts, essential oils are receiving particular attention. They exhibit antibacterial [8], antiviral, antifungal, antiparasitic and insecticide effects [9]. Essential oils have also been shown to have antispasmodic and antioxidant properties [10]. This wide spectrum of biological activities of essential oils is causing a growing interest in their potential use as a control of food deterioration and as natural antioxidant. Moreover essential oil presents lower risk for human health and environnement. They also do not leave residues in food [11].

Our previous investigation on essential oil of *H. cheirifolia* leaves collected in vegetative stage from Oum el bouaghi, a semi-arid region located in Algerian east revealed high content of (-) drimenin [12]. We aimed in the present study to investigate the chemical composition of essential oil of *H. cheirifolia* leaves collected in flowering stage from the same location (Oum el bouaghi). In addition antibacterial and antioxidant activities were evaluated.

2. Experimental

2.1. Plant material

The leaves of *H. cheirifolia* were collected during February 2010 (flowering stage) in Oum el bouaghi, Algeria. The plant was identified by Prof. Zellagui Amar and a voucher specimen was deposited in the Laboratory of Biomolecules and Plant Breeding, University of Larbi Ben Mhidi Oum Elbouaghi under number ZA 122.

2.2. Extraction

Collected leaves (100g) were hydrodistilled in a Clevenger-type apparatus for 3 h. Essential oil was then collected and submitted to GC-MS analysis to determine its chemical composition.

2.3 Chemical composition

Gas chromatography- mass spectrometry : Analysis were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240°C, respectively; oven temperature programmed from 60°C to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection 0.2 μL (10% n-hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of their retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons, and by computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data**.**

2.4. Antibacterial activity

2.4.1.Microbial strains

The antibacterial activity was evaluated by standard strains. The Gram negative bacteria evaluated included *E. coli ATCC25922*, *Pseudomonas aeruginosa ATCC27853* and *K. pneumonia* a clinical strain isolated from patient. The Gram positive bacteria *Staphylococcus aureus ATCC 25923* was also tested.

2.4.2.Agar diffusion method

The antibacterial activity was determined using the agar diffusion method according to the National Committee of Clinical Laboratory Standard (NCLLS) guidelines [13]. Sterile Mueller Hinton (MH) agar was poured into Petri dishes and left to set. Then, Petri plates were inoculated with the microorganism inoculum. The inoculum was prepared with an overnight culture of test microorganism and the size was adjusted to 0.5 McFarland standard turbidity. Sterile paper disk prepared and permeated with 25, 100, 500 and 2000 μg/ml of essential oil of *H. cheirifolia* were placed on to agar plates containing one of the mentioned bacteria. The plates were then left at room temperature for 30 minutes and incubated at 37C° for 24 hours. Antibacterial activity was determined by measuring the diameters of the inhibition zone of essential oil of *H.cheirifolia*. All tests were performed in triplicate in three different experiments**.**

2.5.DPPH radical-scavenging activity

The capacity of extracted essential oil from *H. cheirifolia* to reduce the radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) was assessed using the method described by Masuda *et al.* 1999 [14]. 15 µl of the essential oil at different concentrations was added to 15 µl L of a DPPH ethanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min in the dark. The absorbance of the resulting solution was then measured at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of essential oil was evaluated according to the formula:

$$\text{DPPH}\cdot \text{ scavenging effect } (\%) = (A0 - A1)/A0 \times 100$$

where $A0$ is the absorbance of the control at 30 min, and $A1$ is the absorbance of the sample at 30 min. All samples were analyzed in three replications.

3. Results and discussion:

3.1. Chemical composition of the essential oil

The hydrodistillation of fresh leaves of *H. cheirifolia* gave brown oil with a yield of 0.7 %. GC-MS analysis of the oil has resulted in the identification of 62 compounds representing 78.29 % of the total oil (table1). Compounds belonging to the class of monoterpenoids and sesquiterpenoids represented 33.64% of the total

identified compounds in which oxygenated monoterpenoids and sesquiterpenoids were the dominant compounds in their respective group with 14,23 and 10,83% , respectively.

Table-1: Chemical Composition of *H.cherifolia* leaves essential oil.

Compounds	Rt	%
Cyclopropyl carbinol	2.025	0.78
t-Butylbenzene	2.241	**3.06**
1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-one	2.760	0.60
1-Bromo-2-methylbutane	2.824	0.52
3-Thujanone	3.015	0.76
cis-Verbenol	3.387	1.49
Terpineol	3.892	0.98
α-pinene	4.064	0.78
Terpineol acetate	4.070	0.57
Verbenone	4.245	0.51
p-Decyloxyaniline	8.373	0.10
1,3,6-Heptatriene,2,5,6-trimethyl-	11.276	0.80
β –Caryophyllene	12.06	1.64
Humulene	12.97	0.21
2-Methyl-1-(2-methyl-4-propoxyphenyl)propan-1-one	13.058	0.12
Cyclopropanecarboxylic acid,1-hydroxy-,(2,6-di-t-butyl-4	13.418	0.31
Palustrol	13.742	1.12
Valencene	13.80	Tr
Germacrene D	14.353	0.22
Naphtalene,1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methylethyl)	14.449	0.06
Cadinene	14.57	Tr
Naphtalene,1,2,3,5,6,8a-hexahydro-4.7-dimethyl-1-(1-methyl)	14.578	0.98
Mesitylene, 2-(1,3-butadienyl)-	14.769	0.15
Oxamide,N-(3-methoxypropyl)-N'-cycloheptylidenamino-	14.826	0.10
3-Cyclohexene-1-methanol, α,α,4-trimethyl-	15.192	Tr
Spathulenol	15.359	1.30
5-Hepten-3-one,2-(5-ethenyltetrahydro-5-methyl-2-furanyl)	15.498	1.44
6.ß .Bicyclo[4.3.0]nonane,5. ß.-iodomethyl-1. ß.-iso	15.585	0.45
2-Dodecen-1-yl (-) succinic anhydride	15.727	1.17
1-Isopropenyl-3-propenylcyclopentane	15.866	0.47
4-Propylbenzaldehyde diethyl acetal	15.898	0.26
Selina-6-en-4-ol	15.98	0.51
2-Ethoxyphenethylamine	16.023	0.35
Benzene,(1-ethyldecyl)-	16.267	0.13
(Z,E)-α-Farnesene	16.43	Tr
Bicyclo [4.4.0]dec-1-ene,2-isopropyl-5-methyl-9-methylene	16.494	0.35
Dimethandrostanolone	16.552	0.39
Tricyclo[6.3.0.0(2,4)]undec-8-ene,3,3,7,11-tetramethyl-	16.615	2.49
Aromadendrene, dehydro-	16.84	Tr
1.1,3-Dimethyl-2-(1-methylethylidene)cyclopentane	16.968	0.12
Decane,1-chloro-	17.007	0.15
2,3,4-Trifluorobenzoic acid,tridec-2-ynyl ester	17.58	0.23
3-Tetradecyne	17.71	0.21
22,23-Dibromostigmasterol acetate	17.948	0.49
Bioallethrin	18.03	0.21
Ethanone,1-(1,4-dimethyl-3-cyclohexen-1-yl)-	18.386	0.24
Anthracene,1,2,3,4,5,6,7,8-octahydro-9,10-dimethyl	18.445	0.96
(-)-Drimenin	18.51	**6.71**
2,3,3-Trimethyl-2-(3-methylbuta-1,3-dienyl)-6-methylenecy	18.66	0.40
2H-1-Benzopyran-2-one,6-acetyl-7-(acetyloxy)-4-methyl-	19.071	0.38

Isobutyl phthalate	19.140	0.38
2H-Pyran,2-[(1,1-dimethyl-2-butynyl)oxy]tetrahydro	19.175	0.20
Methanamine,N-(1,3,5-trimethyl-4-pyrazolyl methyl	19.764	0.09
4α,5α-Epoxycholestane	19.912	0.16
Phthalic acid , cyclobutyl tridecyl ester	20.132	0.13
Cyclohexane,1,3,5-trimethyl-	20.614	0.20
Cycloisolongifolene, 8,9-dehydro-9-formyl	21.06	0.86
Borane, oxybisdiethyl-	21.533	0.12
valeranone	23.58	**6.90**
1,1,1,5,7,7,7-Heptamethyl-3,3 bis(trimethylsiloxy)tetrasilox	27.154	0.13
Monoethylhexyl phthalate	29.319	**33.71**
3,4-Dihydroxymadelic acid (tms)	31.291	0.14
Total		**78.29**

Table -1 shows the retention time and the peak area percentage of the identified compounds. The major compound which based on the peak area percentage was monoethylhexyl phthalate (1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) (33.71%), valeranone (6,9%), (-)-Drimenin (6,71%) and benzene, tert-butyl (3,06%). However, Our previous investigation on essential oil of *H. Cheirifolia* leaves collected in vegetative stage from the same area (Oum el bouaghi) revealed high content of (-) drimenin (67,4%), 1,2-di (2-pyridinyl)-1,2-ethanediol (11,2%), globulol (1,7%) and cycloheptane, 4-methylene-1-2-(2-methyl-1-propen1-yl)-1-vinyl-(1,5%) [12]. Previous study on the aerial parts (leaves and stems) of *H. cheirifolia* growing in Téjrouine in Tunisia reported thymol (61%) and 2,6-dimethoxy-phenol (12.83%) as major compounds [6]. Another study concerning Tunisian *H. cheirifolia* growing in Thala reported α pinene (62,5%), germacrene D (9,5%), α-cadinol (2,7%), sabinene (2,1%) and β-caryophyllene (1,7%) as major compounds of the vegetative part (leaves and stems). Concerning flowers the main compounds were α pinene (70,4%), germacrene D (6,7%), α-cadinol (3,2%) and sabinene. While α pinene (22,1%), valencene (13,2%), β-caryophyllene (11,8%), germacrene A (7,8%), α-terpinyl acetate (6,9%), germacrene D (5,9%), β- elemene (3,9%) and caryophyllene oxide (2,5%) were the major compounds of roots [15]. Monoterpene hydrocarbons were the most abundant class in flowers and vegetative parts. While sesquiterpene hydracarbons were dominants in roots [16]. Variation in the chemical composition of distilled essential oils is known to differ considerably not only due to the existence of different subspecies, but might also be attributed to other factors such as: varied agro climatic conditions (climatic, seasonal, geographic) of the region, stage of maturity, adaptive metabolism of plants, distillated conditions, the plant part analyzed and some others factors [17,18].

The most considerable point found among the identified compounds is the presence of phthalate compounds. Moreover 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (mono (2-ethylhexyl) phthalate or MEHP) 33,71% was the major compound in the essential oil of *Hertia cheirifolia.* Two other phthalate compounds namely: 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester (phthalic acid, diisobutyl ester) and phthalic acid , cyclobutyl tridecyl ester were also detected. GC-MS analysis also detected the presence of organosilocon compounds and bioallethrin a pyrethroid insecticide. Detection of phthalate compounds in the essential oil might reveal the presence of contamination. Phthalate contaminations have been previously reported from the essential oils of plants in several studies [19-22]. Mono (2-ethylhexyl) ester phthalate was the main constituents of essential oil of *Senna podocarpa* [23], *Moringa oleifera* [24], *Polygonum chinense* [25], and *Iris germanica* [26] where it was shown to exhibit good antimicrobial activities.

The presence of phthalates in plant might be associated with environmental exposure. Production of phthalates in plants is still in doubt and case of discussion between scientists. Further study dealing with the presence of phthalates in plant will be very interesting and will help to understand whether those compounds are environmental contaminant or they might be considered as a plant metabolite especially in the case of MEHP.

3.2.Antibacterial activity

In general *H.cheirifolia* essential oil displayed varying degrees of antibacterial activity against the tested bacteria. Results of the disc diffusion tests are summarized in table 2. The diameter of the inhibition zone varied from 8.6 ± 1.52 to 23.0 ± 0 mm. *Pseudomonas aeruginosa* ATCC27853 was the most resistant bacteria since the oil showed weak inhibition toward it. 25 µg/ml of the tested oil showed no inhibition, while 2000 µg/ml led to a diameter zone of 12.6 ± 0.15 mm. *P. aeruginosa* was reported to be highly resistant even to synthetic drugs. This ability is due to its restrictive membrane barrier [27]. The tested oil had moderate activity on *Staphylococcus aureus* ATCC25923, *Escherichia coli* ATCC25922 and *Klebsiella pneumonia*. At 2000 µg/ml

H.cheirifolia essential oil seem to act in the same way on the three mentioned bacteria with no significant difference in the diameter of the zone of inhibition.

Our results are in contradiction with researches of Majouli et al (2016). The authors reported a pronounced activity of flowers and vegetative parts (leaves and stems) of *H.cheirifolia* essential oil on Gram positive bacteria especially on *S.aureus* which displayed the strongest effect. The tested Gram negative bacteria (*E. coli, P. aeruginsa and A. baumanii)* were less active with the weakest values for *P. aeruginsa* and *A. baumanii* [16].

There are few reports on the antimicrobial activity of essential oil of the genus *Hertia*. Bammou et al (2016) showed that essential oil of *H. maroccana* had a moderately activity against Gram negative strains (*E. coli and S. atony)* and Gram positive strains *(S. aureus and B. subtilis)* [28]. Akhgar et al (2012) demonstrated that *H.intermedia* essential oil restrained Gram positive bacterial growth. The tested oil was inactive on Gram negative bacteria [29].

Table-2: Antibacterial activity of *H. cherifolia* essential oil.

Strain bacteria	25 µg/ml	100 µg/ml	500 µg/ml	2000 µg/ml
***Staphylococcus aureus* ATCC25923**	**12.3±0.47**	**15.0±0**	**20.5±1**	**22.6±0.15**
***Escherichia coli* ATCC25922**	**12±0**	**18.6±0**	**22.3±1.15**	**23.0±0**
Klebsiella pneumonia	**8.6±1.52**	**9.6±0.15**	**16±0**	**22.33±1.0**
***Pseudomonas aeruginosa* ATCC27853**	**..**	**8,0±0**	**10.3±0.57**	**12.6±0.15**

A wide variety of essential oils are known to possess antimicrobial properties and in many cases this activity is due to the presence of active constituents, mainly attributable to isoprenes such as monoterpenes, sesquiterpenes and related alcohols , others hydrocarbons and phenols [6]. In this study the essential oil of *H.cherifolia* was found to be abundant in phthales derivatives, monoterpenes and sesquiterpenes. Phthales derivatives were the major compounds with 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester as the major compounds. The antimicrobial activity of essential oil is related in most cases into its major components and the synergistic effects of the minor constituents should be taken into consideration. Referring to literature mono (2-ethylhexyl) ester phthalate has shown considerable antimicrobial properties [26, 30].

3.3. DPPH radical-scavenging activity:

The DPHH radical-scavenger activity is one of the most widely accepted and used method for assessing antioxidant activity of plant extracts and essential oil. Assays of essential oil of *H.cheirifolia* were performed using a concentration range of 10^{-1}M, 10^{-2}M, 10^{-3}M and 10^{-4}M. Ascorbic acid was used as standard and positive control (fig.1).

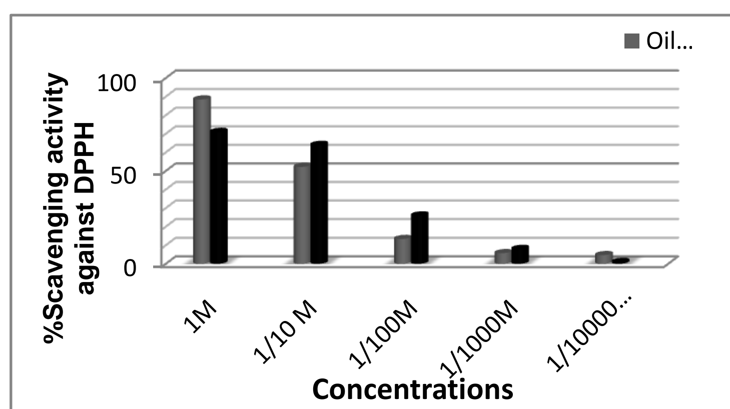

Figure-1: Scavenging activity of essential oils against the DPPH radical at different concentrations.

The highest DPPH radical scavenging activity (%) was shown by essential oil at 10^{-1}M (88.47 %) which was exerted higher antioxidant activity than standard vitamin C (71%).

Our results are in agreement with Majouli K et al. The authors reported a high scavenger ability of *H.cheirifolia* essential oil with DPPH assay. In addition, the tested oil exhibited a strong reducing capacity. Among all tested oils (flowers, leaves + stems and roots), flowers essential oil demonstrated the best capacity of inhibition of lipide peroxidation with value lower than the used control (BHT) [15].

Conclusion

The chemical composition of the hydrodistilled essential oil of *H. cheirifolia*, cultivated at Oum El Bouaghi, was determined by GC/MS. 62 components representing 78,29 % of the essential oil were identified with monoethylhexyl phthalate (1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) (33.71%), valeranone (6,9%), (-)-Drimenin (6,71%) and benzene, tert-butyl (3,06%) as the main compounds. The tested essential oil showed a moderate antibacterial activity and a strong antioxidant activity. Thus, the strongest antioxidant activity of *H. cheirifolia* essential oil may make it a candidate to help to prevent food deterioration. The tested oil can be used as natural antioxidant in food preservation.

In general biological activities of essential oil are related to their composition, nature of chemical structure and their proportion. Further studies are still in need to help understanding the potential uses of *H. cheirifolia* essential oil. The study of the correlation between detected activities and identified compounds presents in the oil will be of great interest. Moreover, the identification of compounds involved in biological activities may enhance the use of of *H. cheirifolia* essential oil.

Acknowledgements-Authors are grateful for the partial financial support by The MESRS (Ministère de l'Enseignement Supérieur et de la Recherche Scientifique) Algeria.

References

1. Nordenstam B., The Biology and Chemistry of the Compositae, Heywood V. H., Harborne J. B., Turner B. L eds, (1977).
2. Beniston N.T., Beniston W.S. Fleurs d'Algérie F.N.1. Alger, (1984).
3. Pottier-Alapetite G. Flore de la Tunisie. Imprimerie Officielle de la République Tunisienne. 11 (1981).
4. Massiot G., Nuzillard J. M., Le men-olivier L., Aclinou P., Benkiouder A., *Phytochem*. 29 (1990) 2207.
5. Ammar S., Edziri H., Mahjoub M.A., Chatter R., Bouraoui A, Mighri Z., *Phytomed*. 16 (2009) 1156.
6. Attia S., Grissa K., Mailleux A., Heuskin S., Lognay G., Hance T., *Pest Manag Scie.* 68 (2012) 1069.
7. Dubey S., Maity S., Singh M., Saraf S. A. S. *Adv. Pharmacol* (2013)1.
8. Benjilali B., Ayadi A. *Plantes médicinales et phytothérapies.* 2 (1986) 155.
9. Burt S. *Inter. J. Food. Microbiol*. 94 (2004) 2
10. Lopes-lutz D., Alviano D. S., Alviano C. S., Kolodziejczyk P. P. *Phytochem*. 69 (2008) 173
11. Fandohan P., Gbenon J. D., Gnonlonfin B., Hell K., Marasas W. F. O., Wingfield M. J. *J. Agric. Food. Chem*. 52 (2004) 6829.
12. Zellagui A., Gherraf N., Lahouel M., *J of New Tech and Mater*. 2 (2012) 38.
13. NCCLS. National Committee for Clinical Laboratory Standards., Approved Standard M100-S12. Wayne PA. (2002)
14. Masuda T., Yonemori S., Oyama Y., Takeda Y., *J. Agric. Food. Chem*. 47 (1999) 1749.
15. Majouli K., Hlila M. B., Flamini G., Ben Jannet H., Kenani A. *Ind. Crops.Prod*. 82 (2016): 23-28.
16. Majouli K., Hlila M. B., Flamini G., Ben Jannet H., Kenani A. *J. Croast Life Med.* 4 (2016) 865.
17. Amirah T., Kamazeri T. S., Abd Samah O., Taher M., Susanti D., Qaralleh H., *Asi. Pac. J. Trop. Med*. (2012) 202.
18. Majoilia K., Besbes Hlilab M., Hamdic A., Flamini G., Ben Jannete H., Kenani A., *Indus Crops & Prod.* 82 (2016) 23.
19. Manayi A., Saeidnia S., Shekarchi M., Hadjiakhoondi A., Shams Ardekani M. R., Khanavi, M., *Res. J. Phamacog*. 1 (2012) 37.
20. Srinivasan G. V., Sharanappa P., Leela N. K., Sadashiva G. T., Vijayan K. K., *Nat prod Rad*. 8 (2009) 488.
21. Di-Bella G., Saitta M., Pellegrino M., Salvo F., Dugo G., *J. Agric. Food. Chem*. 47 (1999) 1009.
22. Manayi A., Kurepaz-mahmoodabadi M., Gohari A., Ajani Y., Saeidnia S., *DARU J of Pharmaceu Scie*. (2014) 22.
24. Zhao S., Zhang D., *Sep and Purif Tech*. 118 (2013) 497.
25. Bagavathi P. E., Ramasamy N., *Pharmacognosy Res*. 14 (2012) 11.
26. Syeda FA., Ur-Rahman H., Choudahry MI., Ur-Rahman A., *Intern J of Gen and Mol Bio.* 3 (2011) 95.
27. Cavar S., Maksimovic M., Vidic D., Paric A. *Indu. Crop. Prod*. 37 (2012) 479.
28. Bammou M., Bahlali E. T., Sellam K., Ibijbijen J., El Rhaffari L., Nassiri L. *J. App. Pharm. Sci.* 6 (2016) 131
29. Akhgar M., Shariatifar M., Akhgar A., Moradalizadeh M., Faghihi-Zarandi A., *Chem. Nat. Comp.* 48 (2012) 329
30. Singh R., Singh S. K., Maharia R. S., Garg A. N., *J Pharm and Biomed Anal*. 105 (2015) 150.

(2017) ; http://www.jmaterenvironsci.com