J. Mater. Environ. Sci. 6 (10) (2015) 2778-2783
ISSN : 2028-2508
CODEN: JMESCN

Determination of some bioactive chemical constituents from *Thesium humile* Vahl.

G. Belakhdar[1], A. Benjouad[1], E.H. Abdennebi[2*]
[1] *Biochemical and Immunological Laboratory, Faculty of Sciences, University of Mohammed V, Rabat, Morocco.*
[2] *Department of Biological and Pharmaceutical Sciences, IAV Hassan II, Rabat, Morocco.*

Received 24 Mar 2015, Revised 15 Sept 2015, Accepted 15 Sept 2015
[*]*For correspondence: E-mail: pr.abdennebi@yahoo.fr / Tel: (+212 6 60 54 60 85)*

Abstract
Thesium humile Vahl is a small herb which parasitizes cereal cultures. It is considered toxic to small ruminants. Our previous research indicated that this plant contains alkaloids in its aqueous extract. One of these alkaloids was identified as the 1-hydroxymethylpyrrolizidine. The present study deals with the identification of other bioactive chemical constituents from *Thesium humile* Vahl by GC-MS analysis. Twenty compounds have been identified by comparing their spectra data to those available in the chemical libraries. These compounds are mainly fatty acid esters, phenols, alkanes and alkenes. The fatty acids and phenols are the more represented. Most of the identified compounds are cited in the literature for their antimicrobial, antifungal, antioxidant, anti-cancer, anti-inflammatory and hypo-cholesterolemic properties.

Keywords: Thesium humile Vahl, Phytochemical constituents, Biological activity, GC-MS analysis.

1. Introduction
Plants produce a wide variety of bioactive compounds with significant applications in different sectors. These compounds occur naturally in small quantities and are considered as secondary plant metabolites with pharmacological or toxicological properties in living organisms [1].
Thesium humile Vahl (Santalaceae) is an obligate hemiparasitic angiosperm plant, endemic in North Africa and probably in other Mediterranean countries [2]. It is an annual herb which parasitizes several host species of Poaceae, Fabaceae and others [3]. In addition of causing important yield losses in cereal crops [4], this plant is also responsible of animal poisoning, especially among small ruminants [5].
Previous phytochemical examination of *Thesium humile* indicated the presence of β-sitosterol and tricosanol–12 from the petroleum extract and an unidentified crystalline flavonol glycoside from the chloroformic extract of its defatted material. Thin layer chromatography (TLC) has also shown the possible presence of the β-stigmasterol [6]. Recently, our phytochemical and toxicological studies on this plant confirm its toxicity to mice and allow the identification of the pyrrolizidine alkaloid 1-hydroxymethylpyrrolizidine, a probable intermediate toxic principle of this herb [7].
In the continuation of our research on *Thesium humile*, this study aim to identify other chemical constituents with potential pharmacological properties, by using gas chromatography-mass spectrometry (GLC-MS).

2. Materials and methods
3. 2.1. Plant material
Aerial parts of *Thesium humile* Vahl were collected in May 2013 from Khemisset-Morocco, where the plant grows intensively and where it has caused poisonings to small ruminants. The identification of the plant was confirmed by the Department of Ecology of the Institute of Agronomic and Veterinary Medicine, Rabat. A voucher specimen (RAB 78257) was deposited at the herbarium of the Scientific Research Institute of Rabat-Morocco.

2.2. Extraction and purification procedures

The extraction and purification procedures followed are those described by El-Shazly *et al*. [8] for the isolation of pyrrolizidines alkaloids. Briefly, 200 g of the defatted powder of the aerial part of *Thesium humile* was dissolved in 2N HCL and filtered. The acidic extract was made alkaline (pH=9) with concentrated ammonia and eluted with dichloromethane through anhydrous sodium sulfate to eliminate any remaining water. The organic solvent was evaporated under reduced pressure to yield a dichloromethane extract. This extract was chromatographed on a silica gel column using n-butanol/acetic acid/water (6/1/1) as the elution mixture. This fractionation gave five main fractions (F1 to F5) based on their TLC pattern (Kieselgel 60 F254, 0.20 mm, Merck), using n-butanol/acetic acid/water (6/3/2). The different fractions were re-dissolved in methanol and analyzed by GC-MS.

2.3. GC-MS analysis and conditions

The analysis was carried out by an Agilent GC-MS (6890N GC and 5973 inert MSD) equipped with a split less injector and a capillary DB-5 MS column [30 m × 0.25 mm × 0.25 µm (Agilent p/n 122-5532UI)]. The injection port temperature was maintained at 285°C and the column oven temperature program was set from 40°C to 240°C (6 /°C min), then increased to 300°C (5°C /min), ending with a 5 min isothermal at 300°C. The carrier gas was Helium (1 ml/min) and the mass spectra were recorded at 70 eV. The chemical components were identified by comparison of their mass fragmentation patterns to these of the reference standard data of WILEY and (NIST) libraries.

3. Results and discussion

TLC of fraction F2 and F3 were positive to Dragendorff reagent, suggesting the presence of compounds with alkaloids type. Their GC-MS analysis, revealed the presence of 1- hydroxymethylpyrrolizidine, a compound which was already identified from this plant [7]. TLC of fraction F1, F4 and F5 did not reveal any spots reacting with Dragendorff. However fraction F4 had many spots visible with either UV light and/or sulfuric vanillin. GC-MS analysis of this fraction showed the total ion chromatogram given in Figure-1.

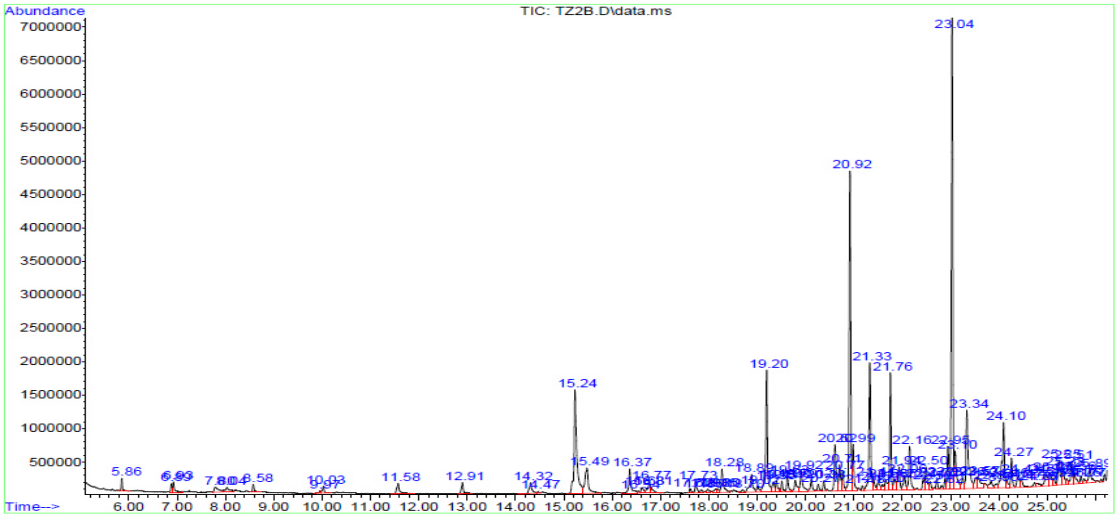

Figure 1: Total ion chromatogram of fraction 4 of *Thesium humile*

From this analysis, a total of twenty compounds have been detected and identified mainly as fatty acid esters, phenols, alkanes, alkenes and aromatic chemicals. Mass spectrums of the five major compounds are given in figures 2 to 6.

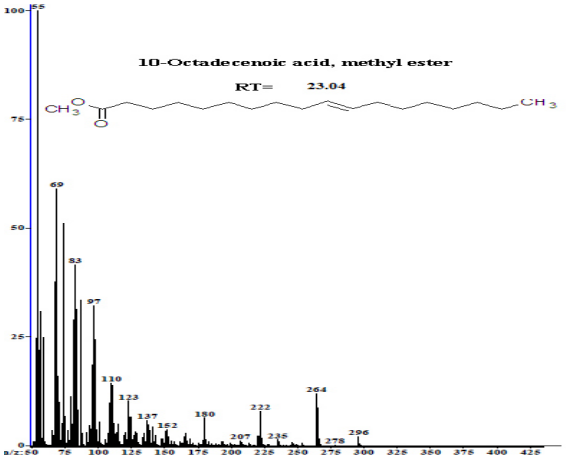

Fig. 2: Mass spectrum of 10-octadecenoic acid methyl ester

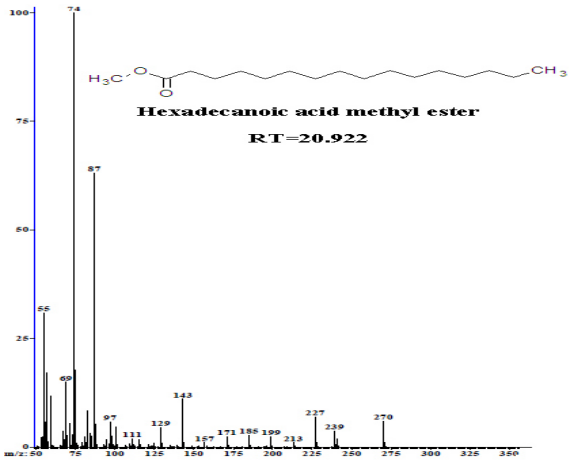

Figure 3: Mass spectrum of hexadecanoic acid methyl ester

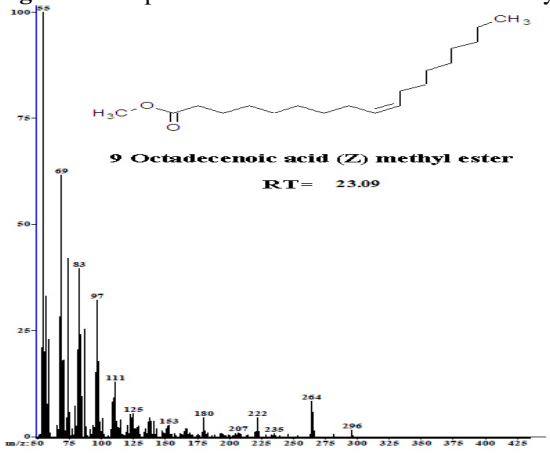

Fig. 4: Mass spectrum of 9-octadecenoic acid (Z) methyl ester

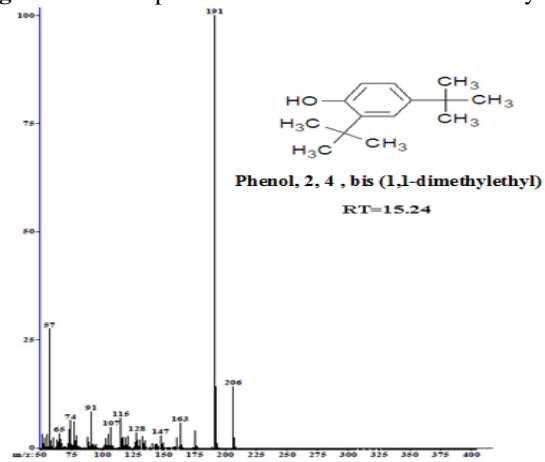

Figure 5: Mass spectrum of phenol, 2,4-bis (1,1-dimethylethyl)

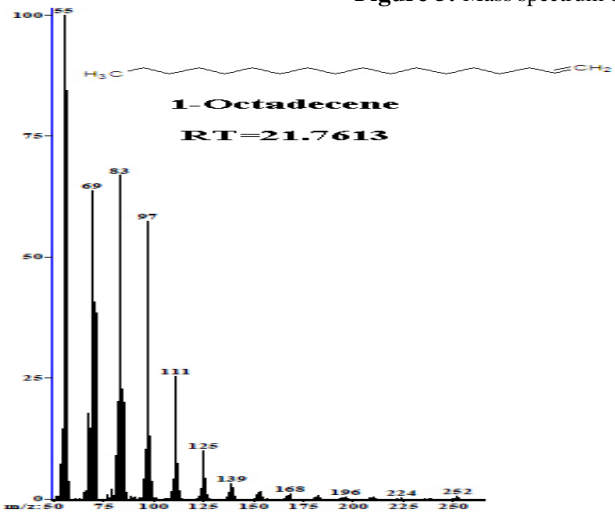

Figure 6: Mass spectrum of 1-octadecene.

Retention times (RT), molecular formulas, molecular weights (MW) and relative concentrations (peak areas %) of these chemicals are reported in table-1.

Table 1: Chemical constituents identified from the fraction 4 of *Thesium humile*

RT (min)	Chemical name	Nature of the compound	Molecular formula	Molecular weight	Peak area (%)
8.04	Undecane	Alkanes	$C_{11}H_{24}$	156.30	0.09
14.96	3-3Dimethyl Hexane	Alkanes	C_8H_{18}	212.41	0.01
15.24	Phenol, 2,4-bis(1,1-dimethylethyl)	Phenol	$C_{14}H_{22}O$	206.17	8 .16
15.25	Dodecanoic acid methyl ester	Fatty acid methyl ester	$C_{13}H_{26}O_2$	214,34	0.09
15.49	Phenol 4,6-di(1,1-dimethylethyl)-2-methyl	Phenol	$C_{15}H_{24}O$	220.35	2.47
16.36	1-Hexadecene	Alkenes	$C_{16}H_{32}$	224.42	2.89
16.86	n- Tridecanoic acid methyl ester	Fatty acid methyl ester	$C_{14}H_{28}O_2$	228.37	0.14
18.28	n-Tetradecanoic acid methyl ester	Fatty acid methyl ester	$C_{15}H_{30}O_2$	242.39	1.52
19.13	Pentadecanoic acid methyl ester	Fatty acid methyl ester	$C_{16}H_{32}O_2$	256.42	0.11
19.20	1-Octadecanol	Fatty alcohol	$C_{17}H_{36}O$	270.49	8.14
19.40	Cyclotetradecane	alkane	$C_{14}H_{28}$	196.22	0.40
20.92	Hexadecanoic acid methyl ester	Fatty acid methyl ester	$C_{17}H_{34}O_2$	270.45	21.25
21.30	1,2 Benzene dicarboxylic acid dibutyl ester	Aromatic compound	$C_{16}H_{22}O_4$	278.00	10.20
21.76	1-Octadecene	Alkene	$C_{18}H_{36}$	252.28	7.95
22.89	Eicosane	Alkane	$C_{20}H_{42}$	282.54	0.47
23.04	10-Octadecenoic acid methyl ester	Unsaturated fatty Acid methyl ester	$C_{19}H_{36}O_2$	296.48	27.45
23.09	9-Octadecenoic acid (Z) methyl ester	Unsaturated fatty acid methyl ester	$C_{19}H_{36}O_2$	296.48	2.20
23.33	Octadecanoic acid methyl ester	Fatty acid methyl ester	$C_{19}H_{38}O_2$	298.50	3.07
24.10	Hexadecanoic acid, butyl ester	Fatty acid butyl ester	$C_{20}H_{40}O_2$	312.00	2.52
24.27	Docosane	Alkane	$C_{29}H_{60}$	408.78	0.11

Two compounds namely 10-octadecenoic acid methyl ester and hexadecanoic acid methyl ester were found to be major in this fraction with 27.45% and 21.25% peak area respectively. Many minor constituents were also identified such as : 1,2-benzenedicarboxylic acid dibutyl ester (10.2%), phenol, 2,4-bis (1,1-dimethylethyl) (8.16%), 1-octadecanol (8.14%), 1-octadecene (7.95%), octadecanoic acid methyl ester (3%), 1-hexadecene (2.9%), hexadecanoic acid, butyl ester (2.5%), phenol 4,6-di (1,1-dimethylethyl)-2-methyl (2.5%), 9-octadecenoic acid (Z) methyl ester (2.2%), tetradecanoic acid methyl ester (1.5 %), etc.

Most of the identified compounds have been reported to possess interesting biological activities (Table 2).

Phenol, 2,4-bis (1,1-dimethylethyl), dodecanoic acid methyl ester, 1-hexadecene, n-tridecanoic acid methyl ester, n-tetradecanoic acid methyl ester, pentadecanoic acid methyl ester, 1-octadecanol, 1,2 benzenedicarboxylic acid dibutyl ester, eicosane, 10-octadecenoic acid methyl ester, and octadecanoic acid methyl ester have both antibacterial and antifungal properties [9-19]. While 1-octadecene and docosane act only on bacteria [20-21].

Several studies have attributed the antioxidant effect to phenol, 2,4-bis (1,1-dimethylethyl), phenol 4,6-di(1,1-dimethylethyl)-2-methyl, 1-hexadecene, hexadecanoic acid methyl ester, 1-octadecene, 10-octadecenoic acid methyl ester, 9-octadecenoic acid (Z) methyl ester and hexadecanoic acid, butyl ester [22-27].

Phenol, 2,4-bis(1,1-dimethylethyl), phenol 4,6-di(1,1dimethylethyl) -2 -methyl, 1-octadecene, eicosane and 9-octadecenoic acid (Z) methyl ester are reported as anti-cancer and/or antitumor active principles [14, 23, 24, 26, 28-30].
Hexadecanoic acid methyl ester and 10-octadecenoic acid methyl ester seem to have the ability to decrease blood cholesterol [12, 24] hexadecanoic acid methyl ester inhibits the cyclooxygenase II enzyms and, thus, produce a selective anti-inflammatory action [24].

Table 2: Reported biological activities of the identified bioactive compounds from *Thesium humile*.

Compound	Biological activity
Phenol, 2,4-bis(1,1-dimethylethyl)	antimicrobial [18], antifungal [9], antioxidant [22], antitumor [29-30],
Dodecanoic acid methyl ester	Antibacterial, antiviral, antifungal [10]
Phenol 4,6-di(1,1-dimethylethyl) -2-methyl	Antioxidant, anticancer [26]
1-Hexadecene	Antibacterial, antifungal, [14,16],antioxidant [25].
n-Tridecanoic acid methyl ester	Antibacterial, antifungal [13].
n-Tetradecanoic acid methyl ester	Antibacterial, antifungal [13].
Pentadecanoic acid methyl ester	Antimicrobial, antifungal [13].
1-Octadecanol	Antibacterial, antifungal, anti-larva [17].
Hexadecanoic acid methyl ester	Anti-oxidant, decrease blood cholesterol, anti-inflammatory [24].
1,2Benzenedicarboxylic acid dibutyl ester	Antimicrobial, antifungal, anti-malarial [15].
1-Octadecene	Antibacterial, antioxidant [20], anticancer [28].
Eicosane	Antibacterial, antitumor, antifungal, cytotoxic [14].
10-Octadecenoic acid methyl ester	Antibacterial, antifungal, antioxidant, decrease blood cholesterol [12].
9-Octadecenoic acid (Z) methyl ester	Antioxidant, anti cancer [23, 24].
Octadecanoic acid methyl ester	Antimicrobial [11].
Hexadecanoic acid, butyl ester	Antimicrobial [19], antioxidant [27].
Docosane	Antibacterial [21].

The antimicrobial and cytotoxic effects related to these substances are due to the fact that fatty acids, fatty acid ester and aliphatic chains (long chain alkanes and alkenes) normally accumulated in the lipid layer of the cell membrane and mitochondria. Consequently, they disturb the integrity of cell structure which becomes permeable [31-32].Unsaturated fatty acids are also known to lower blood cholesterol levels [33] Phenol derivatives also alter cell homeostasis and lead to growth inhibition and cell death [34]. This phenomenon results from the ability of these compounds to alter efflux pumping and, thus, pH, increase membrane permeability [35].

Conclusion

This study reveals that, in addition of being highly toxic to livestock, *Thesium humile* may also be considered as an important medicinal plant. In fact, twenty chemical constituents have been identified from a fraction of the dichloromethane extract of the plant by GC-MS analysis. A high percentage of these compounds is represented by fatty acids and phenol compounds which possess many desirable biological activities.

Acknowledgement - Authors are thankful to the Doping Control Laboratory of the Institute of Agronomy and Veterinary Hassan II of Morocco for providing GC-MS analysis.

References

1. Kris-Etherton P.M., Hecker K.D., Bonanome A., Coval S.M., Binkoski A.E., Hilpert K.F., *Am.J. Med.* 113 (2002):71S.
2. Taleb A., Bouache M., Rzozi S., *Actes Inst. Agron. Vét.* (Maroc) 18 (1998) 121.
3. Chabrolin C.. *Annales du Service Botanique de Tunisie* 11(1934) 65.
4. El Hamri D., *Mémoire d'Ingénieur d'Application*, IAV Hassan II, Rabat, (1988) 72 p.
5. Bellakhdar J.,Hommes et plantes au Maghreb: éléments pour une méthode en ethnobotanique, [Metz] : Plurimondes, cop2008.
6. Samir A., Gharbo, M.R.I., Saleh E.M A., *Planta Med.* 17(1969) 236.
7. Belakhdar G., Benjouad A., Kessabi M., Abdennebi E.H., *J. Mater. Environ. Sci*. 5 (2014) 811.
8. El-Shazly A,Sareg T., Ateya A., Abdel Aziz E., El-Dahmy S., Witte L., Wink M. *J. Nat. Prod*.59 (1996) 310.
9. Rangel-Sánchez G., Castro-Mercado E., García-Pineda E. *J. Plant Physiol*. 17 (2014) 189.
10. Özçelik B., Aslan M., Orhan I., Karaoglu T. *Microbiol. Res*. 160 (2005) 159.
11. Gehan M.A., Hanan A.E., Hassan A.H.I., Okbah, M.A. *World Sci. J*. 7 (2009) 872.
12. Asghar S.F., Choudahry M.I. *Inter. J. Genetics Molecular Biol*. 3 (2011) 95.
13. Chandrasekaran M., Senthilkumar A., Venkatesalu V. *European Rev. Med. Pharmacol*. Sci. 15 (2011) 775.
14. Hsouna A.B., Trigie, M., Mansour R.B., Jarraya R.M., Damak M., Jaoua, S. *Inter. J. Food Microbiol.* 148 (2011) 66.
15. Elija K., Vaishali B., Adsul M.K., Deshpande N.R., Kashalkar R.V. *J. Phar. Res*. 5 (2012)
16. Yogeswari S., Ramalakshmi S.N., Muthu J.M. *Global J. Pharmacol*. 6 (2012) 65.
17. El-Hawary S.S., El-Tantawy M.E., Rabeh M.A., Badr W. K. *Inter. J. Appl. Res. Natural Products*. 6 (2013) 4.
18. Salini T.S, Divakaran D., Shabanamol S., Sharrel R., Jisha M.S.,*World J. Pharm. Res*. 3 (2014) 879.
19. Sujatha, Karthika, Sivakamasundari ,Mariajancyrani, Chandramohan. *Inter. J. Pharma. Chem. Biol. Sci.* 4 (2014) 112
20. Mishra P.M., Sree A. *Asian J. Plant Sci*. 6 (2007) 168.
21. Uma B., Parvathavarthini R. *Inter. J. Pharm. Tech. Res*. 2(2010) 1677.
22. Ajayi G.O., Olagunju J.A., Ademuyiwa O.C., Martins O. *J Med Plant Res* 5 (2011) 1756.
23. Syeda F.A, Habib-ur-Rahman A.M. Khan, Choudahry M.I., Atta-Ur-Rahman. *Inter. J. Genetics Mol. Biol*. 3 (2011) 95.
24. Hema, R., Kumaravel, S., Alagusundaram. *J. Am. Sci*. 7 (2011) 27.
25. Yan Mou, Jiajia Meng, Xiaoxiang Fu, Xiaohan Wang, Jin Tian, Mingan Wang, Youliang Peng, Ligang Zhou. *Molecules* 18 (2013) 15587.
26. Al-Shwyeh Hussah A., Abdulkarim Sabo M., Rasedee A., Elwathig Saeed Mirghani M., Al-Qubaisi M. *BMC Complement Altern Med* 25 (2014):199.
27. Prakash O., Gondwal M., Pant A.K. J. Nat. Prod. Res. 2 (2011) 435.
28. Lee YS,Kang M.H., Cho Y.S., Jeong C.S. *Arch. Pharm. Res*. 30 (2007) 436.
29. Sujana N., Ramanathan S., Vimala V., Sundaram M. *Inter. J. Pharm. Pharmaceutical Sci*. 4 (2012) 17.
30. Panigrahi S., Sundaram Muthuraman M., Natesan R., Pemiah B. *Inter. J. Pharm. Pharmaceutical Sci*. 6 (2014) 93.
31. Hansch C., Dunn W.J. *J Pharm Sci.* 61 (1972) 1.
32. Solorzano-Santos F., Miranda-Novales M.G. *Curr. Opin. Biotechnol. 23* (2012) 136.
33. Okwu D.E., Morah F.N.I., *J. Med. Arom. Plant. Sci*. 28(2006) 605.
34. Devi K.P., Nisha S.A., Sakthivel R., Pandian S.K. *J. Ethnopharmacol.* 130 (2010) 107
35. Srivastava J., Chandra H., Nautiyal A.R, Swinder J. S. K. *Biotech.* 4 (2014) 451.