Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(6): 477-482 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Chemical composition, Antimicrobial activity and chromosome number of *Urospermum dalechampii* from Algeria

Messaoud Ramdani[1*], Takia Lograda[1], Pierre Chalard[2, 3], Gilles Figueredo[4], Heythem Laidoudi[1], Abdelkader Ounoughi[1].

[1] Laboratory of Natural Resource Valorisation, SNV Faculty, Setif 1 University, 19000 Setif, Algeria
[2] Clermont Université, ENSCCF, Institut de Chimie de Clermont-Ferrand, BP 10448, F-63000 Clermont-Ferrand, France
[3] CNRS, UMR 6296, ICCF, F-63171 Aubière, France
[4] LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France

***Corresponding author**
Messaoud Ramdani
Email: ramdanimessaoud@yahoo.com

Abstract: The chemical composition of essential oil, isolated from *Urospermum dalechampii* by hydrodistillation, was analysed by GC and GC/MS. A total 26 compounds representing 97.82% of the oil were identified in Amouchas population. This oil is Characterized by a high rate of palmitic acid (24.3%), henecosane (10.1%), 2-methyl-Z,Z-3,13-octadecadienol (7.1%), tricosane (6.73%), dill apiole (5.25%), myristic acid (4.8%), myristicin (4.7%), 2-pentadenanone-6,10,14-trimethyl (4.4%), lauric acid (3.9%), elemicin (3.3%). Other major compounds are present in the essential oils of this species, isobutyl phlatate, caryophyllene oxide, epicubenol-1, β-guaiene, α-bisabolol and 3,7-dimethyl-octa-1,6-diene. To test the antibacterial activity of essential oil of *U. dalechampii*, eleven bacteria are used in this study. The oil showed a significant effect against Gram-negative bacteria, and modest antibacterial activity against Gram-positive bacteria. The population of *U. dalechampii* studied showed a diploid chromosome number with 2n = 2x = 14, and a basic chromosome number x = 7.
Keywords: *Urospermum dalechampii*, Essential oil, antibacterial activity, Chromosome, Algeria

INTRODUCTION

The *Urospermum dalechampii* displays a similar spectrum of secondary metabolites that *U. picroides* [1-4]. *U. dalechampii* is rich in flavonoids including quercetin aglycone, which is present in all species of the genus [3, 5-7]. The chemical composition of *U. dalechampii* presents the melampolide and guaianolide zaluzanin [3,8-9].

U. dalechampii contains mucilage and phenolic compounds with antioxidant activity and antimicrobial properties [3], effects against gastric problems[10]. The authors found that the *U. picroides* has anti-inflammatory activity [11-12]. This species is a source of indole alkaloids [13], with many properties (angiogenic, anti-oxidant, anti-inflammatory) [11]. The essential oil *U. Dalechampii* has significant antibacterial activity against *Staphylococcus aureus* [14-15].

The genus *Urospermum* in Mediterranean is represented by two species. Studies have shown that *U. picroides* has a single diploid chromosome number 2n = 10 with a basic number of x = 7 [16-25].

The *U. dalechampii* is less studied chromosomally. The only chromosome number cited is 2n = 14 [26-29].

MATERIAL & METHODS

Plant material

Urospermum Dalechampii L. species belongs to the tribe Cichorioideae with a monogamous capitulates. Perennial species with a black strain; stems 10-60 cm, pubescent, simple or rowers, open at the top. The lower leaves are panduriformes; oblong or ovate-lanceolate, entire or toothed. Large flower heads with yellow flowers and purple sulfur outside (Figure 1). The involucres are bracts, broadly lanceolate and pubescent. Akènes bec progressivement atténuées à la base et au sommet. The fruits are black with a long spur and a tuft of hair [30]. This species is distributed in the center of Europe, North Africa and West Asia [31], on uncultivated land, meadows and fields

Urospermum dalechampii is collected from the locality of Amouchas (Setif) in eastern Algeria. Aerial parts were collected during the flowering stage in April 2014. The air dried materials were subjected to hydro-distillation for 3h using a Clevenger apparatus type.

Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Setif University, Algeria. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 mL/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the *m/z* range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library[32-33] and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values[34].

Antibacterial Activity

The Extract Essential oil was tested against the following bacteria; seven gram negative bacteria: *Acinetobacter baumanii* ATCC 19606; *Citrobacter freundii* ATCC 8090; *Escherichia coli* ATCC 25922; *Salmonella typhimurium* ATCC 13311; *Klebsiella pneumoniae* ATCC 700603; *Proteus mirabilis* ATCC 35659; *pseudomonas aeruginosa* ATCC 27853, and five gram positive bacteria; *Bacillus cereus* ATCC 10876; *Bacillus subtilus* ATCC 663313; *Enterococcus faecalis* ATCC 49452; *Lysteria monocytogenes* ATCC 15313 and *Staphylococcus aureus* ATCC 25923. The *in vitro* antibacterial activity of the examined extract was assessed the determination of the activity by the micro dilution method, according to recommendations of the Clinical and Laboratory Standards Institute.

The bacterial inoculums was prepared from overnight broth culture in physiological saline (0.8 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar) and MH agar supplemented with 5 % sheep blood for fastidious bacteria were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution and diluted essential oil (1:1, 1:2 and 1:5 v:v of DMSO). DMSO was used as negative control. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (Bacteria). After incubation, inhibition zone diameters were measured and documented.

Karyology

For karyotypic analysis, the squashing method is used. The root-tip meristems of from germinating seeds were usually used for chromosome preparations. A pre-treatment at room temperature for 2 hours was usually applied before fixation of the root-tips, in a 0.05% water solution of colchicine. After fixation in a cold mixture of ethanol acetic acid (3:1), the root-tips were stored in cold 70° ethanol until used. The following procedure involved the maceration in 45% acetic acid for 15 min. staining of chromosomes is made of emerging root-tips in acetic orcein with heating for one minute. Cutting off the meristems and squashing them in a drop of orcein.

RESULTS

The hydrodistillation of *Urospermum dalechampii* essential oil gave a viscous liquid. The yield of essential oil of the sample is 0.01%. The analysis and identification of components of the essential oil was performed using the (GC-MS). The compounds, identified in this oil and their relative abundance, are presented in their order of appearance (Table 1). The analysis allowed us to identify 26 components, representing 97.82% of total oil of Amochas population.

The chemical composition of the essential oil of *U. dalechampii* is dominated by the presence of the major products, palmitic acid (24.3%), henecosane (10.1%), 2-methyl-Z,Z-3,13-octadecadienol (7.1%), tricosane (6.73%), dill apiole (5.25%), myristic acid (4.8%), myristicin (4.7%), 2-pentadenanone-6,10,14-trimethyl (4.4%), lauric acid (3.9%) and elemicin (3.3%). We also note the presence of components in concentrations greater than 1%, isobutyl phlatate, caryophyllene oxide, epicubenol-1, β-guaiene, α-bisabolol and 3,7-dimethyl-octa-1,6-diene.

The antibacterial activity of the essential oil of *U. dalechampii* is evaluated by the disc method. The results are expressed by the diameter measuring inhibition halos in mm after 24 h incubation at 37°C (Table 2). The action of the essential oil of *U. dalechampii* is very marked on bacteria *Acinetobacter baumanii*, *Staphylococcus aureus* and *Salmonella typhimurium* with halos than that of gentamicin, while the bacteria *Enterococcus faecalis*, *Citrobacter freundii* and *Proteus mirabilis* are resistant to the oils of *U dalechampii* (Figure 2). The essential oil of Amochas population shows moderate activity against *Salmonella typhimurium*, *Bacillus cereus* and *Klebsiella pneumoniae*, while it is weak against the three bacteria *Lysteria monocytogenes*, *Pseudomonas aeruginosa* and *Escherichia coli*.

The study of the metaphase plates of *Urospermum dalechampii* of Amoucha population, allowed us to observed a diploid chromosome number with 2n = 2x = 14 (Figure 3).

Figure 1 : *Urospermum dalchampii* from Amouchas region (Algeria)

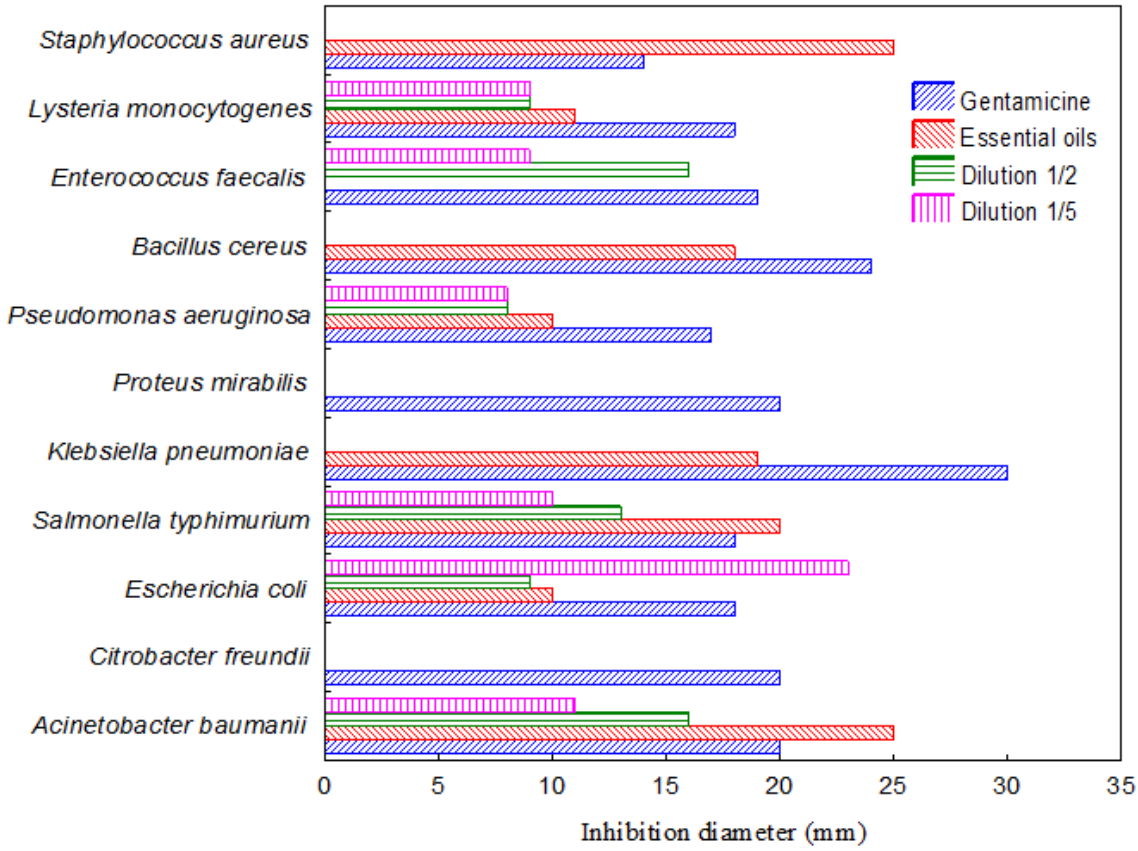

Figure 2: Antibacterial activity of essential oil of *Urospermum dalechampii*

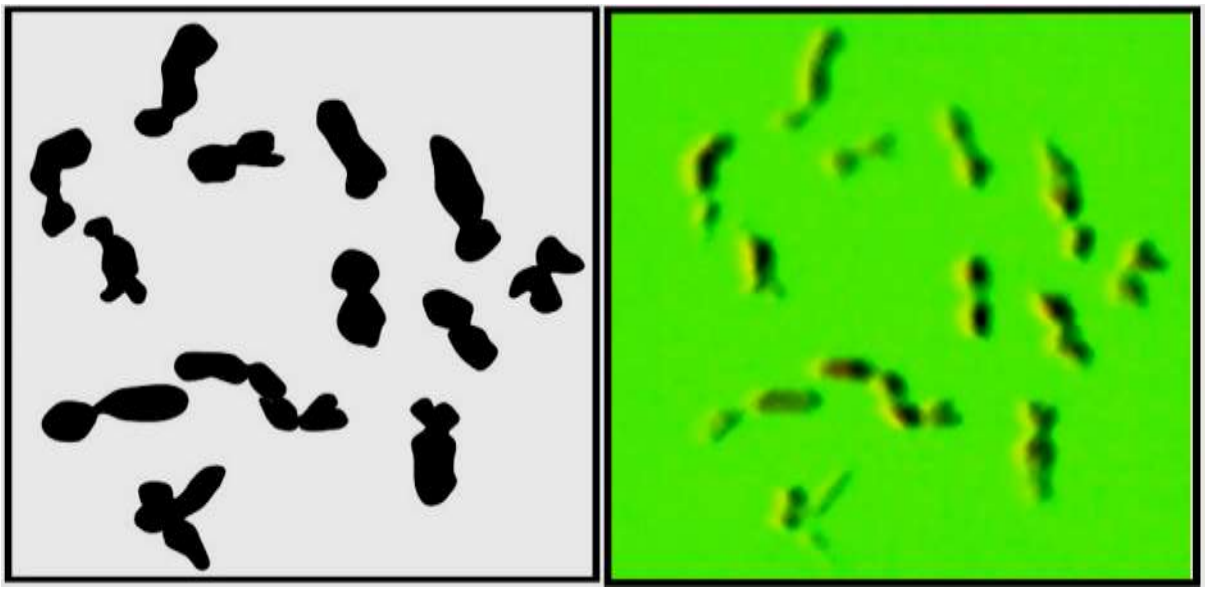

Figure 3: Karyotype of *Urospermum dalechampii* (2n = 2x = 14)

Table 1: Chemical composition of the essential oil of *Urospermum dalechampii*

Yield (v/v)	KI	0.01
Number of compounds		26
Total %		97.82
Camphor	1120	0.16
β-caryophyllene	1428	0.26
Germacrene-D	1480	1.31
Valencene	1491	0.79
Myristicin	1520	**4.70**
Elemicin	1554	3.30
Lauric acid	1570	3.90
Caryophyllene oxide	1573	2.48
Dill apiole	1622	**5.25**
Epi-cubenol-1	1627	2.17
β-guaiene	1663	2.00
Alloaromadendrene oxide-(1)	1671	1.03
α-bisabolol	1683	2.08
3,7-Dimethyl-octa-1,6-diene	1563	2.08
1-Dodecanol, 3,7,11-trimethyl	1570	1.12
2-Pentadecanone-6,10,14-trimethyl	1700	**4.40**
Myristinic acid	1735	**4.80**
trans-Farnesol	1741	0.70
Isobutyl phthalate	1912	2.60
Palmitic acid	2009	**24.30**
Abietatriene	2050	1.81
Heneicosane (n C21H44)	2090	**10.10**
2-Methyl-Z,Z-3,13-octadecadienol	2104	**7.10**
Docosane (n C22H46)	2208	2.05
9-Tricosene-(Z)	2250	0.60
Tricosane (n C23H48)	2307	**6.73**

Table-2: Inhibition diameter of the essential oil of *Urospermum dalechampii*

Bacteria	Gentamicine	*Dilution*		
		1	½	1/5
Acinetobacter baumanii ATCC 19606	20	25	16	11
Staphylococcus aureus ATCC 25923	14	25	0	0
Salmonella typhimurium ATCC 13311	18	20	13	10
Bacillus cereus ATCC 10876	24	18	0	0
Klebsiella pneumoniae ATCC 700603	30	19	0	0
Lysteria monocytogenes ATCC 15313	18	11	9	9
Pseudomonas aeruginosa ATCC 27853	17	10	8	8
Escherichia coli ATCC 25922	18	10	9	23
Enterococcus faecalis ATCC 49452	19	0	16	9
Citrobacter freundii ATCC 8090	20	0	0	0
Proteus mirabilis ATCC 35659	20	0	0	0

DISCUSSION

Few studies of the essential oils of the *U. dalechampii* were performed. The genus *Urospermum* is characterized by the melampolide-type germacranolides [3, 35-36]. To our knowledge no study has mentioned the chemical composition of the essential oils of this species. Our results show the presence of palmitic acid, myristinic acid and other components.

Antibacterial activity of essential oils of the *U. dalechampii* is tested on 11 bacterial strains; the results show that the oil of this species has a significant inhibitory action on almost all the bacteria tested. Cassandra et al.,[38] found that there was a significant activity of extracts from *U. dalechampii* on *S. aureus*. The significant antibacterial activity of the essential oil of *U. dalechampii* is probably due to its high content in the fatty acid, because the linolenic acids, lauric acid, palmitic acid and other fatty acid are powerful on antibacterially of U. dalechampii [39].

Urospermum dalechampii is very homogeneous in terms chromosome number. Our karyotype shows a diploid chromosome number 2n = 14, with a number of base x = 7; this result confirms the previous counts [26-29].

CONCLUSION

This work is focused on the phytochemical study, karyological and evaluation of antibacterial activity of *Urospermum dalechampii*. The analysis of the chemical composition of essential oils by (GC/MS) allowed us to identification 26 components in the oil. The composition of the essential oil is dominated by the presence of the major product palmitic acid. Antibacterial activity of essential oils of the *U. dalechampii* is tested on 11 bacterial strains; the results show that the oil of this species has a significant inhibitory action on almost all the bacteria tested. The observation of metaphase plates of *U. dalechampii*, allowed us to observe a diploid chromosome number 2n = 2x = 14, with a basic chromosome number x = 7.

ACKNOWLEDGEMENTS

The work was supported by VRBN laboratory, Ferhat Abbas University (Sétif 1) and Chemical Laboratory of carbohydrates Heterocyclic of Clermont Ferrant, France.

REFERENCES

1. Amer MMA, Salama OM, Bohlmann F, Ziesche J; Urospermal, a glucoside from *Urospermum picroides*. Phytochemistry, 1984; 23: 692–693.
2. Balboul B A A A, Ahmed AA, Otsuka H; Sesquiterpene lactones and glucosides fromUrospermum picroides. Phytochemistry, 1997; 45: 369–373.
3. Marco JA, Sanz-Cervera JF, Yuste A, Oriola MC; Sesquiterpene lactones and dihydroflavonols from Andryala and Urospermum species. Phytochemistry, 1994; 36: 725-729.
4. Salam NAA, Mahmoud ZF, Ziesche J, Bohlmann F; A glucoside of urospermal A., Phytochemistry, 1982; 21: 2746–2747.
5. Giner RM, Recio MC, Cuellar MJ, Manez S, Peris JB, Stubing G, Mateu I, Rios JL; A Taxonomical study of the subtribe *leontodontinae* based on the distribution of phenolic compounds. Biochem. Syst. Ecol., 1993; 21: 613–616.
6. Rios JL, Giner RM, Cuellar MJ, Recio MC, Serrano A; 1992. Phenolics from some species of subtribe Leontodontinae. Planta Med, 1992; 58: (Suppl. 1): 701.
7. Sareedenchai V, Zidorn C; Flavonoids as chemosystematic markers in the tribe Cichorieae of the Asteraceae, Biochem. Syst. Ecol., 2009; 1-23.
8. Bentley RK, Buchanan JGSC, Halsall TG, Thaller V; Urospermal A and urospermal B, conformers of a germacranolide aldehyde from *Urospermum dalechampii*, Journal of The Chemical Society D: Chemical Communications, 1970; 7: 435-436.
9. Rychlewska U, Hodgson DJ, Grabarczyk H, Drozdz B, Daniewski WM, Kroszczynski W, Budesinsky M, Holub M; Sesquiterpene lactones of

Urospermum dalechampii Schmidt. Collect. Czech. Chem. Commun, 1986; 51: 1698-1709.
10. Gastaldo P; Compendio della Flora Officinale Italiana. Piccin, Padova. 1987.
11. Strzelecka M, Bzowska M, Kozie J, Szuba B, Dubiel O, Rivera Nunez D, Heinrich M, Bereta J; Anti-inflammatory effects of extracts from some traditional Mediterranean diet plants (Conference paper), Journal of Physiology and Pharmacology, 2005; 56: 139–156.
12. Manez S., Recio M.C., Giner R.M., Sanz M.J., Terencio M.C., Peris J.B., Stubing G., Rios J.L., 1994. A chemotaxonomic review of the subtribe crepidinaebased on its phenolic constituents. Biochemical Systematics and Ecology, 22: 297–306
13. Ji X, Lu G, Gai Y, Zheng C, Mu Z ; Biological control against bacterial wilt and colonization of mulberry by an endophytic *Bacillus subtilis* strain. FEMS Microbiol. Ecol., 2008; 65: 565–573.
14. Atzei AD; Le piante nella tradizione della Sardegna. Sassari, Italy: Carlo Delfino ed. (in Italian), 2003; 319-323
15. Quave CL, Plano LRW, Bennet BC; Quorum Sensing Inhibitors of Staphylococcus aureus from Italien medicinal plants. Planta Medica, 2011; 77: 188–195
16. Kuzmanov B, Jurukova P; Reports [in Love, A. (ed.) IOPB Chromosome numbers reports LVIII], Taxon, 1977; 26: 557–565.
17. Kuzmanov B; Chromosome numbers of bulgarian angiosperms: An introduction to a chromosome atlas of the Bulgarian flora, Flora Mediterranea, 1993; 3: 19–163.
18. Amal A, Abd El-Wahid, Magda I. Soliman, Reda M. Rizk and Rehab M. Rizk; Cytogenetical studies on Achene colour polymorphism of *Picris asplenoides* L. and *Urospermum picroides* L. (*Asteraceae*) in Egypt, Pakistan Journal of Biological Sciences, 2009; 12(7): 565–573.
19. Vogt Robert, Abelardo Aparicio; Chromosome numbers of plants collected during Iter Mediterraneum IV in Cyprus, Bocconea, 1999; II: 117–169.
20. Vogt Robert, Christoph Oberprieler; Chromosome numbers of North African phanerogams. VIII. More counts in *Compositae*., Willdenowia, 2008; 38: 497–519.
21. Pastor J; Números 257-262, In Números cromosómicos para la flora española. 257-300. Lagascalia, 1983; 12: 117–119.
22. Smalla M; Studies in the *Compositae* of the Arabian Peninsula and Socotra -6. The Hypochaeridinae (*Lactuceae*) in the Arabian Peninsula. Willdenowia, 2000; 30: 315–337.
23. Strid A, Andersson IA; Chromosome numbers of Greek mountain plants. An annotated list of 115 species. Bot. Jahrb. Syst., 1985; 107: 203–228.
24. Malallah G A, Masood M, Al-Dosari M; Chromosome numbers of the Kuwaiti flora III, Willdenowia, 2001; 31: 411–418.
25. Kamel EA; Cytotaxonomical investigations of the Egyptian Compositae (Asteraceae): I-Cardueae and Cichorieae. Compositae Newslett, 2004; 41: 9–28.
26. Capineri R, D'Amato G, Marchi P; Numeri cromosomici per la Flora Italiana. 534–583. Inform. Bot. Ital, 1978; 10: 421–465.
27. Love A, Love D; In: IOPB chromosome number reports LXXVI. Taxon, 1982; 31: 583–587.
28. Luque T, Zarco C R, Devesa J A; Números 321-330. In Números cromosómicos para la flora española. 300-364. Lagascalia, 1984; 12: 286–290.
29. Dahlgren R, KarIsson T, Lassen P; Studies on the flora of the Balearic IsIands. I. Chromosome numbers in Balearie angiosperms. Bot. Not, 1971; 124: 249-268.
30. Quézel P, Santa S; Nouvelle Flore de l'Algérie et des régions désertiques méridionales. 2 Tome, Ed CNRS, Paris, 1963; 1170p.
31. Lack HW, Leuenberger B; Pollen and taxonomy of *Urospermum* (*Asteraceae*, Lactuceae).Pollen and Spores, 1979; 21: 415–425.
32. Masada Y, Analysis of essential oils by Gas Chromatography and Mass Spectrometry. *J. Wiley & Sons, Inc. New York*, 1996.
33. NIST; Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, vers. 2.0. fiveash data, USA., 2002.
34. Adams RP; Identification of essential oils components by Gas Chromatography-Mass spectroscopy, Allured Publishing Corporation Carol Stream, Illinois USA. 2001.
35. Bremer K; Asteraceae: Cladistics and Classification. Timber Press, Portland, 1994.
36. Zidorn Christian, Sesquiterpenoids as chemosystematic markers in the subtribe *Hypochaeridinae* (*Lactuceae*, *Asteraceae*), Biochemical Systematics and Ecology, 2006; 34: 144-159
37. Zidorn Christian; Sesquiterpene lactones and their precursors as chemosystematic markers in the tribe Cichorieae of the Asteraceae, Phytochemistry, 2008; 69: 2270–2296
38. Cassandra Quave L., Lisa Planob R W, Traci Pantuso, Bradley Bennett C; Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*, Journal of Ethnopharmacology, 2008; 118: 418–428.
39. Manning Robert; Fatty acid composition of pollen and the effect of two dominant fatty acids (linoleic and oleic) in pollen and flour diets on longevity and nutritional composition of Honey Bees (*Apis mellifera*), Thesis of Doctor of Philosophy, School of Biological Sciences and Biotechnology, BSc (Hons) Murdoch University, Australia, 2006; 217.