Applied Biology in Saharan Areas *Vol. 1, N. 2, p. 8-15, December 2017*
ISSN: 2571- 9823

In vitro antifungal activities of essential oil of *Saccocalyx satureioides* Coss. et Dur. on fungal species development

Khaldi Achraf [1*], Meddah Boumedien[1,2], Moussaoui Abdellah[1],
Sonnet Pascal[3], Benmehdi Houcine [4]

[1] Laboratory of valorization of vegetal Resource and Food Security in Semi Arid Areas, South West of Algeria, University Tahri Mohamed , Bechar, 08000 Bechar .Algeria
[2] Laboratory of Bioconversion, Microbiology Engineering and Sanitary Security, University of Mascara, Algeria
[3] Laboratory Glycochemistry, of antimicrobials and Agroressources (LG2A), University of Picardie, Amiens France
[4] Faculty Nature and Life Sciences, Department of Biology, University Tahri Mohamed . Bechar, B.P 417, 08000 Bechar

*Corresponding author: achrafsystemdz@yahoo.fr

Abstract – The essential oil of *Saccocalyx satureioides* Coss. et Dur. grows in South-West of Algeria obtained by hydrodistillation and analysed by GC and GC/MS. The major components of oil are α-terpinéol (32.65%) and thymol (22.26%). The essential oil was subjected to the antifungal activities using seven fungi. The results of direct contact method showed that the oil was active against mycelial growth of fungal. All strains were inhibited at minimum inhibitory concentrations (MICs) as from1/880 v/v. *Fusarium oxysporum f.sp. albedinis*, *Alternaria alternata* MTCC 2724 and *Cladosporium species* MTCC 1003 were found to be more sensitive, being inhibited at MIC as weak as 1/1660 v/v. In the other hand, the tested essential oil against the majority of fungi showed total inhibition of the sporulation at concentration as from 1/370 v/v and partial spores' germination with a very high rate.

Keywords: Essential oil, *Saccocalyx satureioides* Coss. et Dur., Antifungal activity, GC-MS.

---

1. Introduction

In the last years a recrudescence of the fungal infections related to the increase in the number of immunocompromised subject has been noted. This recrudescence is accompanied by an increase in the number of identified pathogenic species and emergence of resistance in previously sensitive species (Senhaji and al., 2006). Fungal contamination of a substrate or a food causes physical and chemical modifications, due in particular to the production of pigments. This contamination of the foodstuffs intended for the man or the animal, is the principal damage that will lead to many problems (Tabuc, 2007).

Lately there has been a renewed interest for the medicinal and aromatic plants and their extracts, the face of increased mistrust caused by the use of the chemicals, both in the therapeutic field and food industry (Senhaji and al., 2006).

Recent studies showed that essential oils and their components have an important potential as antimicrobial agents and in several industrial and medical fields (Dorman and Deans, 2000).

Algeria, for its geographical location, offers a rich and various vegetation. *Saccocalyx satureioides* Coss. et Dur.

(Lamiaceae), an endemic Algerian plant which grows in pre-desert areas has been studied. It has been known for its aromatic odor, locally named ̏zaatar rmel and azir l'bel̏ recalling the odor of thyme. In the folk medicine, the aerial part is commonly used in decoction for treatment of gastric disorders and spasms (Ozenda, 2004). This work approaches the characterization of antifungal activities of their essential oil. The chemical composition of the EO also evaluates in this study.

2. MATERIALS AND METHODS

2.1 Plant material

The aerial parts of *S. satureioides* Coss. et Dur. collected in March 2011, in Mecheria, near Naâma Department (South-West of Algeria). A voucher specimen of the plant was identified and authenticated at the laboratory of the biology Institute, University of Tahri Mohamed Bechar. The plant material was dried in a dry and shady place at ambient temperature.

2.2 Extraction of the Essential Oil

The EO of each collective sample isolated from fresh plant material (200g) by hydrodistillation, for 4h, using a Clevenger-type apparatus. The essential oils obtained, dried over anhydrous sodium sulphate and stored in hermetically sealed coloured vials at 4°C before analysis.

2.3 Gas chromatography and Mass spectroscopy analysis

A Hewlett-Packard HP6890 gas chromatograph equipped with a DB-5 fused silica capillary column (30m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific), a FID detector regulated with 260°C, supplied with a mixture of gaz H2/Air and a Split-splitless injector regulated with 240°C used to determine the percentage of oil components. The mode of injection is Split (split ratio: 1/50, flow: 66 ml/min). The gas used nitrogen with a flow rate of 1.7 ml/min. The temperature of the column programmed from 60 to 325°C at a rate of 4 °C/min. Retention indices calculated using the retention time of n-alkanes that injected after the oil at the same chromatographic conditions. Identification of the oil components based on their indices of Kováts (IK) and by comparison of their mass spectral fragmentation patterns with those reported in the literature (Adams, R.P., 2007), and mass spectra, obtained from the GC–MS analysis on a Hewlett-Packard HP6890/HP5973 instrument equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J&W Scientific); helium used as carrier gas at a flow rate of 2 ml/min. The GC analytical parameters are the same as those listed above, and the mass spectrometry runs in the electron impact (EI) at 70 eV.

2.4 Antifungal activities

2.4.1 Fungal strains

The fungal, (*Aspergillus niger* MTTC 2425, *Aspergillus flavus* MTTC 2799, *Aspergillus ochraceus* CECT 2092, *Penicillium expansum* MTTC 1344, *Fusarium oxysporum f. sp. albedinis*, *Alternaria alternata* MTCC 2724 and *Cladosporium species* MTCC 1003), used to evaluate the antifungal properties of the EO. They belong to the fungus collection of biology laboratory of Tahri Mohamed Bechar University. They are cultivated on nutritive medium PDA (*potato dextrose agar*) during seven days in the darkness and at room temperature 25°C. To prepare spore suspension, the fungi grown spores suspended in 0.85% (w/v) sodium chloride to prepare the homogeneous spore suspension (Danmek *et al.,* 2014), using

Malassez hematimeter in order to obtain the concentration of 10^5 spore/ml.

2.4.2 Antifungal activity of EO on mycelial growth

The minimum mycelial growth inhibitory concentration (MIC) of EO measured according to the contact direct method. Because of none-miscibility of EO with water and thus the culture medium, emulsification performed with a 0.2% agar solution (Amarti and al., 2010). The dilutions of 1/10, 1/15, 1/37, 1/150 and 1/750 prepared in the agar solution. In test tubes each one containing 13,5 ml of solid medium PDA, autoclaved for 20 min at 121°C and cooled to 45°C, aseptically added 1.5 ml of each dilution in order to obtain final concentrations 1/100, 1/150, 1/370, 1/1500 and 1/7500 (v/v). Controls contain culture medium and 0.2% agar solutions free of EO are also prepared. After agitation, the selected solutions transferred into Petri plates which inoculated with sporal solution, the incubation carried out at 25°C for 7 days. The antifungal index of mycelial growth in percent (Im %) calculated by the following formula: (Im %) = [(DT- D) / DT] ×100. Where D: mean diameter of mycelial growth of the test and DT: mean diameter of mycelial growth in control (Singh and al., 2009).

For determination of minimal fungicidal concentration (MFC), a fungal disk (5 mm diameter) from each inhibited fungal petri dishes re-inoculated on fresh medium after washing with distilled water and revival of their growth (fungistatic/fungicidal) observed after 7 days. The lowest concentration preventing revival of fungal growth takes as the MFC (Kumar and al., 2008).

2.4.3 Antifungal activity of EO on spore sporulation

From the dishes used for the evaluation of the mycelial growth on solid medium, incubated at 25°C for 10 days, each strain for various concentrations take four washers 5mm diameter and transposed into tubes contain 1ml of sterile distilled water. The Fungal suspension is then agitated using a vortex mixer to release the spores of the conidiophores. The total counts number of spores using a Malassez cell at a rate of 10 counts per suspension. The values are expressed as number of spores per unit area (mm^2) (Serghat and al., 2004). The percentage inhibition of sporulation (Is %) was determined by the following formula: (Is %) = $[(N_0 - Nc) / N_0] \times 100$. N_0 is mean number of the spores estimated in control and Nc is mean number of the spores estimated in the presence of EO.

2.4.4 Antifungal activity of EO on spore germination

The sporal suspension collected was adjusted to 10^5 spores/ml of distilled water using a Malassez cell. 0.1 ml of the suspension sporal plated on petri dishes containing PDA medium to which oils are incorporated at the same concentrations as above with three repetitions performed simultaneously by concentration. Counting spores germinated or not were performed on a total of 200 spores after 18 hours of incubation at 25°C in the dark. A spore was considered germinated if length of the germinatif tube is higher than its smaller diameter (Maouni and al., 2001). The percentage inhibition of germination (Ig %) determined by the following formula: (Ig %) = $(N_0- Nc) / N_0 \times 100$. Where N_0 is mean number of germinated spores in

control and Nc is mean number of germinated spores in the presence of EO.

2.5 Statistical analysis

All determinations for antifungal activities and yield of EO conducted in triplicates and data expressed as mean ± standard deviation. Statistical analysis was performed using SPSS 11.0 Bivariate Correlation Analysis (SPSS Inc., Chicago, IL, USA.). A significant difference was considered at the level of $P< 0.05$ and $P< 0.01$. The Pearson rank correlation test was used for comparisons between the broth dilution and different methods used for antifungal activities.

3. Results and discussion

3.1 Yield and Chemical composition

EO of *S. satureioides* Coss. et Dur. has a yellow color and a strong aromatic phenolic odour. It registered a higher yield as 2.41±0,1% (w/w) based on dry weight, this result is higher than that quoted by Zerroug and al. (2011) with (1.5%) and lower to those obtained by Gourine and al. (2012) along (3.3 %).This may be due to the pedoclimatic conditions of the plant. The yield and quality of EO depends on the geographical origin and the vegetative cycle of the plant, the season of harvest, method and conditions of the extraction.

It is only relative (Satrani and al., 2007).

The chromatographic analysis GC and GC/MS of EO allowed the identification of 33 components representing 94.05% of the total EO content identified (Table 1). This chemical composition is relatively similar to that of El Masrane, area of Djelfa (Center of Algeria) which contained 42 compounds representing 94.1% of the total EO and mainly composed by the α-terpineol (35.9%), thymol (15.6%) and borneol (12.4%), followed by p-cymene (7.2%), camphene (4.3%) with the presence of γ-terpinene (4.0%) (Zerroug and al., 2011). In contrast, the chemical composition of EO is different from that studied by Gourine and al. (2012) which contains as the main constituents as carvacrol (51.82%), γ-terpinene (18.96%) and o-cymene (11.3%).

Table 1. Chemical composition of *S. satureioides* Coss. et Dur. essential oil

N°	IK [a]	compounds	Ss Area %	N°	IK	compounds	Ss Area %
1	926	tricyclene	00.11	18	1285	Bornyl acetate	00.45
2	931	α-thujene	00.20	**19**	**1290**	**thymol**	**22.26**
3	937	1R-α-pinene	01.52	**20**	**1298**	**carvacrol**	**02.79**
4	**953**	**camphene**	**03.00**	21	1355	thymol acetate	00.83
5	980	β-pinene	00.17	22	1409	α-gurjenene	00.10
6	991	myrcene	00.27	23	1418	β-caryophyllène	00.41
7	1018	α-terpinene	00.28	24	1454	α-caryophyllène	00.10
8	**1026**	**p-cymene**	**03.62**	25	1461	Allo-aromadendrene	00.17
9	1030	R-limonene	00.79	26	1493	viridiflorene	00.10
10	1062	γ-terpinene	00.85	27	1505	α-amorphèene	00.19
11	1088	α-terpinolene	00.18	28	1524	Δ-cadinene	00.35
12	1098	linalool	00.25	29	1576	(-)-spathulenol	00.71
13	1139	L-camphor	00.18	30	1581	caryophyllene oxide	00.22
14	**1156**	**isoborneol**	**08.15**	31	1590	viridiflorol	00.28
15	**1165**	**borneol**	**11.04**	32	1653	α-cadinol	00.21
16	1177	terpin-4-ol	01.49	33	1689	shyobunol	00.13
17	**1189**	**α-terpineol**	**32.65**				
Total							94,05 %

[a] Kováts indices calculated on DB5 column with reference to n-alkanes injected after the oil at the same chromatographic conditions.

3.2 Antifungal activities of EO

The antifungal activities of the EO as shown in Table 2. The results of direct contact method of *S. satureioides* Coss. et Dur. EO showed a stronger activity against mycelial growth of the moulds. All strains inhibited at MICs as from1/880 v/v. *Fusarium oxysporum f.sp. albedinis*, *Alternaria alternata* and *Cladosporium species* are found to be more sensitive, being inhibited at MIC as weak as 1/1660 v/v. Also, have noted that *A. ochraceus* inhibited from 1/1150 v/v. However, MFC of 1/370 v/v found fungicide in all tested strains.

On the other hand, the EO show, *in vitro*, an important antifungal activity against germination and sporulation spores'. Only 1/370 v/v of this concentration sufficient to inhibit sporulation for all fungal strains. *Fusarium oxysporum f.sp. albedinis*, *Alternaria alternata* and *Cladosporium species* most sensitive, being inhibited as from 1/1500 v/v. In fact, the degree of inhibition of the EO tested on germination is different from the one exerted on mycelial growth. Higher rate of inhibition observed at concentration of 1/100 v/v against *A. ochraceus* (89.88** %).

Table 2. Antifungal activities of EO from *S. satureioides* Coss. et Dur. expressed in (%) with MICs (v/v).

		Aspergillus flavus	*Aspergillus ochraceus*	*Aspergillus niger*	*Fusarium oxysporum f.sp. albedinis*	*Penicillium expansum*	*Alternaria alternata*	*Cladosporium species*
Control	**Im**	0	0	0	0	0	0	0
	Is	0	0	0	0	0	0	0
	Ig	0	0	0	0	0	0	0
1/7500 (v/v)	**Im**	29,41±0.1	5±0.3	40±0.2	25,39±0.2	21,81±0.5	9,09±0.1	9,09±0.1
	Is	31,14±0.3	20,97±0.2	20,93±0.5	26,54±0.1	34,86±0.6	42,23±0.2	24,57±0.1
	Ig	1,15±0.3	14,04±0.4	4,18±0.5	6,38±0.2	5,23±0.6	2,6±0.3	8,37±0.2
1/1500 (v/v)	**Im**	76,47 **	68,75 *	76,47 *	100 ***	56,36±0.4	100 ***	100 ***
	Is	57,22 *	43,87±0.6	66,74 *	100 ***	52,24±0.4	100 ***	100 ***
	Ig	8,67±0.4	23,03±0.3	35,6±0.4	29,78±0.2	30,15±0.5	21,35±0.3	30,72±0.2
1/370 (v/v)	**Im**	100*** (MFC)	100*** (MFC)	100*** (MFC)	100*** (MFC)	100*** (MFC)	100*** (MFC)	100*** (MFC)
	Is	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***
	Ig	38,15±0.3	39,32±0.4	41,36±0.4	40,42±0.3	38,62±0.4	36,97±0.3	52,51±0.2
1/150 (v/v)	**Im**	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***
	Is	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***
	Ig	56,06±0.2	57,3±0.3	55,49±0.3	50,53±0.4	66,13±0.2	52±0.1	79,88±0.1
1/100 (v/v)	**Im**	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***
	Is	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***	100 ***
	Ig	80,34 ±0.1	89,88 **	75,91±0.2	65,42±0.2	85,71 *	83,85 *	89,38 **
MIC (v/v)		1/880	1/1150	1/880	1/1660	1/880	1/1870	1/1870

* Significant at $p < 0.05$; ** significant at $p < 0.01$; ***significant at $p < 0.001$ according to controls.

Im: antifungal index of mycelial growth; **Is**: percentage inhibition of sporulation;
Ig: percentage inhibition of germination; **MFC**: minimal fungicidal concentration.
Each value in the table obtained by calculating the average of three experiments ± standard deviation.

This importance bioactivity of EO from *S. satureioides* Coss. et Dur. is due to their major's contents of terpene alcohols and phenolic compounds such as α-terpineol (32.65%) and thymol (22.26%), these latter are known for their antimicrobial activity (Dorman and Deans, 2000). Studies by the World Health Organization (1999) shows that thymol has a strong antifungal activity against many species, including *Aspergillus sp.*

Many studies underline the effectiveness of antifungal terpene alcohols and phenols. These results are in agreement with other research that has shown that thymol and α-terpineol are among the most active compounds of EO against fungi due to their high solubility in water and this gives them a high ability to penetrate the walls of fungal cells (El Ajjouri *et al.,* 2008). Against fungi, terpene phenols in EO cause several damage such as morphological disruption of mycelial hyphae, the rupture of the plasma membrane and alteration of mitochondrial structure (De Billerbeck *et al.,* 2001). According to Farag and al. (1989), the presence of OH groups in the phenolic compound is capable of forming hydrogen bonds with the active sites of enzymes and increases the antimicrobial activity. Anyway, the mechanism of phenol toxicity is based on the inactivation of fungal enzymes containing SH group in their active site (El Ajjouri and al., 2008). Furthermore, alcoholic and phenolic terpenes also act by binding to the amine and hydroxylamine groups of microbial membrane proteins causing the deterioration of the permeability and leakage of intracellular components (Lopez-Malo *et al.,* 2005).

However, terpene alcohols and phenols are not only responsible for the activity but all of the chemical composition must be taken into account. Senhaji and al. (2006) reported that minor compounds also play an important role in the activity of EO that seems to act synergistically with the major compounds. Otherwise, we must note that the bioactivity of EO from *S. satureioides* Coss. et Dur. could be due to the synergistic activity between thymol (22.26%) and carvacrol (2.79%). The synergy between these two phenols has been observed in several studies (El Ajjouri *et al.,* 2008). Some studies showed that the antimicrobial activity of EO may be greater than that of the majority of compounds tested separately (Lahlou, 2004). The dominance of the major components in the EO confirms the synergy that could make the minor components in the activity of EO (Lahlou, 2004; Franchomme, 1981).

Indeed, Oussalah and al. (2007) showed that EO inhibits spore germination, elongation of the mycelium, sporulation and toxin production of fungi. The inhibitory activity of α-terpineol (32.65%) also was demonstrated by Belaiche and al. (1996) on the spores' germination of *A. flavus*, *A. parasiticus* and *A. niger*.

4. Conclusion

The overall results of the present work suggest that the EO of S. satureioides Coss. et Dur. can be considered an alternative as a potential source of antifungal agents. It can be used to an antifungal overcoat against the strain that a major problem of fungal infections.

This shows that the Algerian flora may be an important subject of interesting plant species, the active ingredients can be used

in several fields such as food and pharmaceutical industries. Further studies will also be needed to ascertain how to use the selected EO, either alone or in combination with other already existing antifungal compounds.

5. Acknowledgements

The authors would like to thank the pedagogical Biology laboratories of the Bechar University for their help in obtaining the fungal strains used in this investigation and for providing the necessary facilities.

6. References

Adams, R.P., 2007. Identification of essential oil components by gas chromatography/mass spectrometry, 4th Ed. Allured Publishing Co. Carol Stream, Illinois.

Amarti, F., Satrani, B., Ghanmi, M., Abdellah, F., Abderrahman, A., Lotfi, A., Mustapha, E., Abdelaziz, C., 2010. Composition chimique et activité antimicrobienne des huiles essentielles de *Thymus algeriensis Boiss. Reut*. et *Thymus ciliates (Desf.) Benth*. du Maroc. Biotechnol. Agron. Soc. Environ, 14(1), 141-148.

Belaiche, T., Rutledge, D., Ducauze, C., Tantaoui-Elaraki, A., 1996. Inhibition de la germination des spores d'*Aspergillus* par les huiles essentielles. Industries alimentaires et agricoles, vol. 113, n°9, pp. 663-665 (12 ref.).

Danmek, K., Intawicha, P., Thana, S., Sorachakula, C., Meijer, M., Samson, R.A. 2014. Characterization of cellulase producing from *Aspergillus melleus* by solid state fermentation using maize crop residues. African Journal of Microbiology Research, 8(24): 2397-2404.

De Billerbeck, V.G. et al. 2001. Effects of *Cymbopognon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. Can. J. Microbiol, 47, 9-17.

Dorman, H.J.D., Deans S.G., 2000. Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. J. Appl. Microbiol, 88, 308-316.

El Ajjouri, M., Satrani, B., Ghanmi, M., Aafi, A., Farah, A., Rahouti, M., Amarti, F., Aberchane, M., 2008. Activité antifongique des huiles essentielles de *Thymus bleicherianus Pomel et Thymus capitatus (L.) Hoffm & .Link* contre les champignons de pourriture du bois d'œuvre. Biotechnol. Agron. Soc. Environ, 12(4), 345-351.

Farag, R.S., Daw, Z.Y., Hewedi, F.M., El-Baroly, G.S.A., 1989. Antimicrobial activity of some Egyptian spice essential oils. J. Food Prot, 52, 665-667.

Franchomme, P., 1981. L'aromatologie à visée anti-infectueuse. Phytomed 1 and 2, 25-45.

Gourine, N., Benabed, K.H., Ouinten, M., Yousfi, M., 2012. Chemical Composition and Antioxidant Activity of *Saccocalyx satureioides* Essential Oil. Abbstract Book (ISBN 978-9957-31-012-7). The 3rd International Symposium on Medicinal Plants, Their Cultivation and Aspects of Uses. Petra – Jordan, November 21-23, p.149.

Kumar, A., Shukla, R., Singh, P., Prasad, C.S., Dubey, N.K. 2008. Assessment of *Thymus vulgaris L.* essential oil as a safe botanical preservative against post harvest fungal infestation of food commodities. Innov. Food Sci. Emerg. Technol, 9: 575–580.

Lahlou, M., 2004. Methods to study phytochemistry and bioactivity of essential oils. Phytother. Res, 18, 435-48.

Lopez-Malo, A., Alzamora, S.M., Palou, E., 2005. *Aspergillus flavus* growth in the presence of chemical preservatives and naturally occurring antimicrobial compounds. Int. J. Food Microbiol, 99, 119-128.

Maouni, A., Lamarti, A., Douria, A., Badoc, A., 2001. Effet de dérivés calciques sur le développement de moisissures lors de la conservation des poires. Bull. Soc. Pharm. Bordeaux, 140, 79-88.

Oussalah, M., Caillet, S., Saucier, L., Lacroix, M., 2007. Inhibitory effects of selected plant essentail oils on four phathogen bacteria growth : *E.coli* O157H7, *Salmonella Typhimurium, Staphylococcus Aureus* and *Listeria Monocytogenes.* s.l. : Food control, pp. 414-420.

Ozenda, P., 2004. Flore du Sahara septentrional et central. 3th Ed., CNRS, Paris, France.

Satrani, B., Ghanmi, M., Farah, A., Aafi, A., Fougrach, H., Bourkhiss, B., Bousta, D., Talbi, M., 2007. Composition chimique et activité antimicrobienne de l'huile essentielle de *Clanthus miixtus.* Bull. Soc. Pharm. Bordeaux, 146, 85-96.

Senhaji, O., Faid, M., Kalalou, I., 2006. Etude du pouvoir antifongique de l'huile essentielle de cannelle. Phytother, 1, 24-30.

Serghat, S., Mouria, A., Ouazzani touhami, A., Badoc, A., Douria, A., 2004. Effet de quelques fongicides sur le développement in vitro de *Pyricularia Grisea* et *Helminthosporium oryzae*. Bull. Soc. Pharm. Bordeaux, 143, 7-18.

Singh, P., Kumar, A., Dubey, N.K., Gupta, R., 2009. Essential Oil of *Aegle marmelos* as a Safe Plant-Based Antimicrobial Against Postharvest Microbial Infestations and Aflatoxin Contamination of Food Commodities. J. food sci, 74 (6), 302-307.

Tabuc, C., 2007. Flore fongique de différents substrats et conditions optimales de production des mycotoxines. Thèse de doctorat. École Nationale Vétérinaires de Toulouse Laboratoire Biologie Animale, IBNA Balotesti, Spécialité : Pathologie, Mycologie, Genetique et Nutrition, l'institut National Polytechnique de Toulouse (L'INP Toulouse), 27-42, 71-74.

World Health Organization (WHO), 1999. Monographs on selected medicinal plants. Geneva, Switzerland: OMS.

Zerroug, M.M., Laouer, H., Strange, R.N., Nicklin, J., 2011. The Effect of Essential Oil of *Saccocalyx Satureioides Coss. Et Dur*. On the Growth of and the Production of Solanapyrone a by *Ascochyta Rabiei* (Pass.) Labr. ISSN 1995-0756. Adv. Environ. Biol, 5(2), 501-506.