CHEMICAL COMPOSITION AND ANTIMICROBIAL EFFECTS OF TUNISIAN *RUTA CHALEPENSIS* L. ESSENTIAL OILS

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ABSTRACT: The chemical composition of essential oils extracted from different fresh parts of *Ruta chalepensis:* flowers, leaves and stems, was analysed using GC and GC-MS. Fifty three components were identified in the oil of each organ. Ketones occurred as the major constituent of the three organs volatile oils while nonan-2-one was the most notably dominating compound in stems essential oil and undecan-2-one was the major compound in flowers and leaves essential oils. The antimicrobial activity of the indicated volatile fractions was screened against eight bacterial species: *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa ATCC 27853*, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB 8166, *Escherichia coli* ATCC 35218, *Salmonella thyphymerium* LT2, *Listeria monocytogenese* ATCC 19115 and *Enterococcus faecium* 29212 using both disc diffusion and dilution methods. Their antifungal activity was tested against nine fungal species *Aspergillus niger* (onion), *Alternaria solani* (potato), *Penicillium digitatum* (orange), *Botrytis cinerea* (tomato), *Fusarium sambucium* (potato), *Fusarium solani* (potato), *Trichoderma harzianum* T_1 *(soil)*, *Trichoderma viride* T_V (soil*)* and *Fusarium oxysporum* F_{33} (potato). So that leaves essential oil was more active than stems and flowers ones, it shows an important antifungal activity against *Trichodrema viride* T_V. In addition, antimicrobial effects of previously prepared essential oils have been investigated against fifteen Candida species. Thus, all volatile fractions presented significant effects against *Candida albicans* ATCC 90028.

Keywords: *Ruta chalepensis*, Essential oil, Chemical composition, GC and GC-MS, Antibacterial and Antifungal activities.

RESUME: La composition chimique des huiles essentielles issues des différentes parties de *Ruta chalepensis* fraîchement cueillies : les fleurs, les feuilles et les tiges, a été analysée par la chromatographie en phase gazeuse (CG) et la chromatographie en phase gazeuse couplée à la spectrométrie de masse (CG-SM). Cinquante-trois composants ont été identifiés dans l'huile de chaque organe. Les cétones sont majoritaires dans les huiles essentielles des différents organes de la plante. Le nonan-2-one domine l'huile extraite à partir des tiges tendis que le undecan-2-one est le constituant majoritaire des huiles essentielles des fleurs et des feuilles. Les trois fractions volatiles ont subi des tests d'activité antimicrobienne. Elles sont testées contre huit bactéries et neuf moisissures. L'activité antibactérienne a été réalisée selon les méthodes de diffusion et de dilution, en utilisant les bactéries suivantes: *Staphylococcus epidermidis* CIP 106510, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Micrococcus luteus* NCIMB 8166, *Escherichia coli* ATCC 35218, *Salmonella thyphymerium* LT2, *Listeria monocytogenese* ATCC 19115 et *Enterococcus feacium* 29212. L'activité antifongique est effectuée contre les fungicides suivants: *Aspergillus niger* (onion), *Alternaria solani* (potato), *Penicillium digitatum* (orange), *Botrytis cinerea* (tomato), *Fusarium sambucium* (potato), *Fusarium solani* (potato), *Trichoderma harzianum* T_1 (soil), *Trichoderma viride* T_V (soil) et *Fusarium oxysporum* F_{33} (potato). L'huile essentielle des fleurs de *Ruta chalepensis* est plus active que celles des tiges et des feuilles, elle présente une activité antifongique modérée contre *Trichodrema viride* T_V. D'autre part, une évaluation des effets antimicrobiens des huiles essentielles des différents organes a été envisagée à l'encontre de quinze souches de Candida. Les trois échantillons d'huiles ont montré des effets significatifs vis-à-vis de *Candida albicans* ATCC 90028.

Mots clés: *Ruta chalepensis*, huille essentielle, Composition chimique, CG et CG-SM, Activités antibactérienne et antifongique.

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INTRODUCTION

Ruta chalepensis L. belongs to Rutaceae family; it is commonly known as "ruda". Its flowers are cymes with 4-5 sepals, 4-5 petals, 8-10 stames and a superior ovary. *This* perennial herb is widely diffused in the Mediterranean area [1] usually growing on rocky slopes. *Ruta chalepensis* is characterised by glabrous, alternate bi-pennatisect leaves narrow oblong-lanceolate or obovate segments and cymose inflorescence.

This plant was introduced in America after the Spanish conquest [2]. It is one of the most frequently used plants for medicinal purposes [3-4]. Oil glands are principally present in leaves, having strong deterrent odours [5-6]. *Ruta chalepensis* L. has pleiotropic pharmacological properties, attributed to the high content of alkaloids [7-8-9], furocoumarins [7], coumarins [9], furoquinolone alkaloids [10], flavonoids, phenols, amino acids and saponins found in the leaves and young stems of the plant [11]. *Ruta Chalepensis* L. is used in the folk medicine for the treatment of a large variety of diseases.

In Saudi Arabia [1], a decoction of the aerial parts of the plant is used as an analgesic and antipyretic and for the treatment of rheumatism and mental disorders. The leaves of this plant infused with vinegar are given to children for the treatment of convulsion and other nervous disorders. An aqueous decoction of the leaves is used for the treatment of fever in Africa [4]. In addition to it's described emmenagogue, abortificient, antihelmintic and spasmolytic effects [12]. *Ruta Chalepensis* L. also has anti-inflammatory properties [13]. It was active toward *Giardia lamblia* with $IC_{50} < 38$ µg/ml [14]. *Ruta chalepensis* L. has antifungal activity against dermatophytes [15], antifertility, anticonvulsant and sedative effects [16, 17].

The chemical composition of *Ruta chalepensis* essential oil was studied by many teams of researchers in Iran [18], Greece [19], Turkey [20] and India [21-22].

As a part of our contribution to the chemical and biological studies of Tunisian medicinal plants [23-30] and within the aim of valorising medicinal *Ruta chalepensis* cultivated in Tunisia, we report for the first time the investigation of the chemical composition of Tunisian *Ruta chalepensis* flowers, leaves and stems essential oils.

The antibacterial and antifungal effects of the indicated volatile oils were screened against eight bacterial and nine fungal species. Antimicrobial effects against some Candida yeasts were additionally studied and gave significant results.

RESULTS AND DISCUSSION

Table I summarizes the composition of *Ruta chalepensis* essential oils. Three samples were analysed by GC and GC-MS. Qualitatively, the chemical composition of stems, leaves and flowers essential oils are rather close, being dominated by ketones (68.0-80.4%) followed by monoterpenes (4.3-23.1%). We note, however, that the composition presented a slightly difference from stems oil to flowers one.

In fact, 2-undecanone is the most predominant compound found in leaves and flowers (34.7 and 72.0%, respectively). While 2-nonanone is the major component in stems essential oil. Diterpenoids and sesquiterpenes were rarely detected in three samples. Table II, summarizes the antibacterial properties of the obtained essential oils against eight bacteria. Flowers essential oil was active against *Micrococcus luteus* NCIMB 8166 at a concentration of 7µL/disc, while stems and leaves essential oils appeared to be slightly active. The three tested samples have a limited activity against *Staphylococcus aureus* ATCC 25923. We notice that *Escherichia coli* ATCC35218, *Salmonella thyphimirium* LT2, *Pseudomonas aeruginosa* ATCC 27853 were resistant against three indicated oils. This result agrees to the nature of this strain recognized as multiresistant against many antibiotics and biocides agents [31].

According to the results given in table III, the essential oils of *Ruta chalepensis* exhibited very strong antibacterial effects against all cocci Gram positive and reds Gram negative bacteria

except *Pseudomonas aeruginosa* ATCC 27853. Moreover, activity was more effective against Gram positive than Gram negative bacteria.

The results of our bioassays showed that the leaves and flowers essential oils exhibited strong antibacterial activity (MIC: 1-25 µg.mL^{-1}). Flowers essential oil of *Ruta chalepensis* was more active against *Salmonella thyphimirium* LT2 and *Listeria monocytogenese* ATCC 19115, presenting an important growth inhibition at lower concentration (MIC: 1µg.mL^{-1}; MBC: 20 µg.mL^{-1}). Essential oils obtained from leaves and flowers were the most active against *Micrococcus luteus* NCIMB 8166, *Escherichia coli* ATCC 35218 and *Enterococcus faeciu*m 29212 (MIC: 1 µg.mL^{-1}; MBC: 10-20 µg.mL^{-1}).

In the preliminary studies carried out to evaluate antifungal effects of *Ruta chalepensis* essential oils, it was found that flowers volatile fraction exhibited a slight antifungal activity against *Penicillium* sp., while it appeared to be active against *Trichoderma viride* Tv at a concentration of 7µL/Disc (Table IV).

In the other hand, *Alternaria solani, Botrytis cinerea*, *Fusarium sambucinum*, *Fusarium solani* and *Trichoderma harzianum* T_1 were not inhibited by the stems, leaves and flowers essential oils. Further evaluation of the antimicrobial effects of the previously indicated essential oils showed interesting results. In fact, flowers oil was more active against *Candida parapsilosis* ATCC 220129 and *Candida parapsilosis* 1625 than stems and leaves essential oils. We noticed that the three samples were more active against *Candida albicans* ATCC 90028, *Candida albicans* (1605-1613-1630-1672), than amphotericin at a concentration of 7µL/Disc.

MATERIAL AND METHODS

1- Plant Material: *Ruta chalepensis* collected in June 2007 from Jemmel (Monastir, Tunisia) was identified according to the Tunisian Flora [32]. The plant was dried in a natural ventilated place. Fresh organs were separated in three parts: flowers, leaves and peels. Leaves and stems were cut in four little peaces and weighed before essential oil extraction.

2- Volatile Compounds Extraction: Flowers, leaves and stems essential oils were obtained by hydrodistillation of 150g of each considered organ in an overflow flask of Clevenger type during five hours. The essential oil was collected and stored in a refrigerator at 4-5 °C until future use.

3- Analysis of the Essential Oils: Two methods were used in order to analyze the oils:

Gas Chromatography (GC-FID): The identification of the chemical composition of essential oils has been determined on the basis of retention indices using a HP 5890 II GC equipped with an ionization detector of flame (FID) and an apolar capillary column HP-5 which was characterized by 30m of length, 0.25mm of diameter and 0.52µm film thickness. Its stationary phase was constituted of 5% of biphenyl and 95% of dimethylpolysiloxane and a HP polar column INNOWAX of 30m, 0.25mm and 0.25µm film thickness. Its stationary phase is constituted of polyethylene glycol. The injector temperature and the detector are 250°C and 280°C, respectively. Nitrogen is the gas vector (flow rate of 1.2mL/min); the pressure of work is of 9Psi. The apolar column was programmed from 50°-280°C at the rate of 5°C / min with two isothermal points, one at 50°C for 1min and the second at 280°C for 20min. The injected volume was 1µL (dilute to 1% in the hexane). Retention indices (IR) were determined with C_9 to C_{31} alkane standards as reference [20]. Quantitative analysis is achieved by using a software HP chemstation. All coefficients of answers are supposed near. The percentages of the different constituents are assimilated therefore to the percentages of the areas of chromatogram peaks.

Gas Chromatography-Mass Spectrometry (GC-MS): The identification of the oil composition of the plant (diluted to 1% in the hexane) was achieved by coupling GC/MS which was composed by a chromatograph in gaseous phase(HP 5890 II set) coupled to a quadripolar mass spectrometer of (HP-MSD 5972 TO) in electronic impacts. Two types of columns have been used: an apolar capillary column HP5MS, 30m, 0,25mm and 0.25µm film thickness. Its stationary phase was 5% of biphenyl and 95% of dimethyl polysiloxane and a HP polar column INNOWAX 30m, 0.25mm and 0.25µm of film thickness. The conditions of programming were the following: the temperatures of the injector, the source and the interface were 250°C, 175°C and 280°C, respectively. Helium was the gas vector (flow rate: 1.2mL/min), at a pressure of 9Psi. The temperature of the apolar and polar columns was programmed as follows, respectively: 50°C (1min), 50°-280°C at 5°C/min, 280°C (20min) and 50°C (1min), 50°-250°C at 5°C/min, 250°C (20min.).

The injected volume was 1µL (1% in the hexane). The acquirement of the spectral data is achieved in fashion of sweep (2 SCAN/ses), it is from50 to 550 uma and the source is in fashion of electronic impact. The energy of broadcast is 70eVolts. The spectroscopic analysis of the compounds is achieved by comparison with their counterparts with the help of the NBS75K.L spectrotheques and WILLEY275.L. The confirmation has been made by the calculation of the retention indices and comparison with those of the literature [33-34].

4- Biological tests:

Antibacterial assay disc-diffusion method: Antibacterial activity of the oils was screened against eight human pathogenic bacteria (Table II). The strains were obtained from Pasteur Institute collection 106510. The inhibitory effect on bacterial growth was determined using agar-disc diffusion assay [35-36]. The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37°C for 18 to 24h prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bioMerieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bioMerieux) [37-38]. The inocula of the respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37°C during 15min. A sterile filter disc having 6mm of diameter (Whatman paper N°3) was placed at the surface of MH agar and 7µL of the essential oil was dropped onto each Whatman paper disc [39]. The treated Petri dishes were incubated at 37°C for 18 to 24 h. The antibacterial activity was evaluated by measuring the clear zone surrounding the Whatman paper. Standard discs of the antibiotic gentamicin were applied as a positive antibacterial controls.

Antibacterial assay dilution method: The minimal inhibitory concentration (MIC) of oils was determined using the Mueller Hinton broth (MHB) dilution method [40]. All tests were performed in MHB supplemented with DMSO 5% [41]. Bacterial strains were cultured overnight in MHB at 37°C. Tubes of MHB containing various concentrations of oils were inoculated with 10 µL bacterial inoculums adjusted to 10^6-10^7 CFU/mL. They were incubated under shaking conditions (120 rpm) at 37°C for 24h [42-43]. Control tubes without tested samples were essayed simultaneously. The essays were performed in triplicate. The MIC was defined as the lowest concentration preventing visible growth [44-45]. To detect minimal bactericidal concentration (MBC) [40], a lapful 10µL of tested samples were streaked at the surface of TSA agar medium discs; in order to visualize no bacterial growth. The discs were incubated at 37°C during 18 to 24h [46]. The minimum bactericidal concentration (MBC) was determined as the concentration where 99.9% or more the initial inoculums are killed [40, 46]

Antifungal assay disc-diffusion method: The biological activity against yeasts was determined by employing disc agar diffusion method using Sabouraud Dextrose agar [47]. An aliquot (7µL) of the oil was deposited on sterile paper discs (6mm diameter) which were subsequently placed in the centre of the inoculated Petri dishes. After an incubation period of the 24h at 30°C, the inhibitory activity was compared to that of commercial amphotericin at a concentration of 10mg/ml. *Candida* tested were human pathogenic strains obtained from Farhat Hached Hospital Laboratory of Parasitological and Mycology, Sousse Tunisia. The fungal species tested were provided by the Laboratory of Phytopathology of The Regional Center of Research in Horticulture and Organic Agriculture of Chott-Marien (CRRHAB), 4042 Chott-Mariem, 37 Sousse, Tunisia.

Table I. Chemical composition (%) of different parts essential oils of *Ruta chalepensis* L.

No	Compounds	RI apolar	RI polar	Stems	Leaves	Flowers	identification
1	Hex-3-en-1-ol	860	1378	tr	tr	0.1	MS.RI
2	Heptan-2-one	892	1138	tr	tr	tr	MS.RI
3	α-Pinene	940	1032	tr	0.1	tr	MS.RI
4	Myrcene	991	1161	0.3	tr	0.2	MS.RI
5	Octan-2-one	992	1285	tr	tr	0.1	MS.RI
6	Octan-2-ol	1002	1387	tr	tr	0.2	MS.RI
7	(*E*)-3-Hex-2-enyl acetate	1004	1315	tr	tr	0.2	MS.RI
8	*(Z)*- Hex-3-enyl acetate	1009	1320	2.4	3.3	3.0	MS.RI
9	Para-Cymene	1025	1280	0.2	0.1	0.1	MS.RI
10	Limonene	1032	1228	tr	tr	0.2	MS.RI

No	Compounds	RI apolar	RI polar	Stems	Leaves	Flowers	identification
11	1,8-Cineole	1034	1213	tr	tr	0.1	MS.RI
12	*(E)*-β-Ocimene	1048	1252	0.5	tr	tr	MS.RI
13	Nonan-2-one	1094	1392	38.0	30.3	6.9	MS.RI
14	Nonan-3-ol	1094	1470	0.8	1.1	tr	MS.RI
15	Nonan2-ol	1098	1524	0.3	1.2	0.4	MS.RI
16	Nonanal	1105	1399	1.8	8.4	tr	MS.RI
17	Geijerene	1144	1259	tr	0.2	1.1	MS.RI
18	Camphor	1145	1518	tr	0.2	0.7	MS.RI
19	2-Octyl acetate	1149	1497	tr	0.1	tr	MS.RI
20	Nonanol	1177	1664	0.1	1.2	tr	MS.RI
21	Naphtalene	1179	1724	0.1	0.5	0.4	MS.RI
22	Terpinen-4-ol	1179	1612	1.7	0.2	0.1	MS.RI
23	Decan-2-one	1192	1482	6.9	3.2	1.9	MS.RI
24	Methyl chavicol	1205	1665	0.3	0.1	0.3	MS.RI
25	Octyl acetate	1215	1472	11.9	3.2	tr	MS.RI
26	Pulegone	1248	1662	0.1	0.2	tr	MS.RI
27	Decanol	1272	1743	tr	tr	tr	MS.RI
28	Pregeijerene	1288	1359	tr	tr	tr	MS.RI
29	Undecan-2-one	1295	1585	25.6	34.7	72.0	MS.RI
30	Nonyl acetate	1315	1575	0.3	0.3	tr	MS.RI
31	Undecanol	1374	1823	0.2	0.5	tr	MS.RI
32	Dodecan-2-one	1393	1688	0.7	0.6	1.3	MS.RI
33	Decyl acetate	1420	1670	0.6	0.8	tr	MS.RI
34	*β*-Caryophyllene	1427	1608	tr	1.2	1.5	MS.RI
35	Tridecan2-one	1455	1792	3.1	1.8	0.1	MS.RI
36	α-Humulene	1461	1668	tr	0.2	tr	MS.RI
37	Dodecanol	1478	1972	tr	0.2	2.2	MS.RI
38	*(E,E)*-α-Farnesene	1496	1758	0.5	tr	0.2	MS.RI
39	Cis-γ-Bisabolene	1515	1773	tr	0.8	0.2	MS.RI
40	δ-Cadinene	1523	1775	tr	tr	0.4	MS.RI
41	Elemol	1549	2069	tr	0.7	0.3	MS.RI
42	*(E)*-Nerolidol	1566	2030	tr	0.9	0.1	MS.RI
43	*γ*-Eudesmol	1628	2167	0.2	tr	0.2	MS.RI
44	*β*-Eudesmol	1647	2233	tr	0.2	tr	MS.RI
45	*α*-Eudesmol	1650	2188	tr	0.8	0.6	MS.RI
46	Tetradecanol	1676	2168	tr	tr	tr	MS.RI
47	Curcuphenol	1715	2278	tr	0.2	0.1	MS.RI
48	Isophytol	1944	2278	tr	tr	1.1	MS.RI
49	Hexadecanoic acid	1957	2887	tr	tr	tr	MS.RI
50	Phytol	2118	2603	tr	tr	tr	MS.RI
51	Methyl octadecanoate	2128	2412	tr	0.1	tr	MS.RI
52	Ethyl octadecanoate	2195	2460	tr	0.1	tr	MS.RI
53	Phytyl acetate	2221	2652	1.0	tr	1.5	MS.RI
	Alcools			1.5	4.1	2.8	

Ketones	68.0	68.0	80.4
Esters	2.8	3.7	3.4
Aldehydes	1.8	8.4	tr
Acids	tr	tr	tr
Terpenoids	24.1	14.3	8.0
Monoterpenes hydrocarbons	1.1	0.9	0.9
Monoterpenes oxygenated	22.0	8.1	3.4
Sesquiterpenes hydrocarbons	0.7	2.3	2.3
Sesquiterpenes oxygenated	0.3	3.0	1.4
Diterpenoids	1.1	0.22	2.7
Total identified	99.3	98.7	98.3

tr: trace (<0.1%)
RI: retention indices are determined on apolar and polar columns using the homologous series of n-alkanes

Table II. Antibacterial effects of *Ruta chalepensis L* oils disc-diffusion method

Bacteria / Samples of oils	**Stems**	**Leaves**	**Flowers**	**Gentamicin**
Staphylococcus epidermidis CIP 106510	-	7	7	21/10µg
Pseudomonas aeruginosa ATCC27853	-	-	-	22/10µg
Staphylococcus aureus ATCC 25923	8	7	8	24/10µg
Micrococcus luteus NCIMB 8166	10	8	13	27/10µg
Escherichia coli ATCC35218	-	-	-	24/10µg
Salmonella thyphymerium LT2	-	-	-	21/10µg
Listeria monocytogenese ATCC19115	8	-	8	18/10µg
Enterococcus faeacium 29212	-	-	8	26/200µg

-: Not active

Table III. Antibacterial effects of *Ruta chalepensis* L. oils-dilution method

Bacteria / Organs	**stems**		**Leaves**		**Flowers**	
	MIC ($\mu g.mL^{-1}$)	MBC ($\mu g.mL^{-1}$)	MIC ($\mu g.mL^{-1}$)	MBC ($\mu g.mL^{-1}$)	MIC ($\mu g.mL^{-1}$)	MBC ($\mu g.mL^{-1}$)
S. epidermidis CIP 106510	25	ND	25	ND	20	ND
P. aeruginosa ATCC27853	ND	ND	ND	ND	ND	ND
S. aureus ATCC 25923	30	ND	25	ND	10	ND
M. luteus NCIMB 8166	25	ND	1	10	1	10
E. coli ATCC35218	50	ND	1	20	1	20

S. thyphymerium LT2	50	ND	25	ND	1	20
L. monocytogenese ATCC19115	25	ND	25	ND	1	20
E. faecium 29212	50	ND	1	20	1	10

MIC: Minimal inhibitory concentration
MBC: Minimal bactericidal concentration
ND: Not determined

Table IV. Antifungal effects of *Ruta chalepensis* L. oils

	Diameter of inhibition zone (mm)			
Fungal species/ Samples of oils	Stems	Leaves	Flowers	Carbendazim
Aspergillus niger (onion)	7	-	-	17
Alternaria solani (potato)	-	-	-	-
Penicillium digitatum (orange)	-	-	9	24
Botrytis cinerea (tomato)	-	-	-	24
Fusarium sambucium (potato)	-	-	-	-
Fusarium solani (potato)	-	-	-	-
Trichoderma harzianum T_1(soil)	-	-	-	20
Trichoderma viride T_V (soil)	8	-	11	20
Fusarium oxysporum F_{33} (potato)	-	8	-	17

-: Not active

Table V. Antifungal human activity of *Ruta chalepensis* L. oils

Ref	**Origin**	**Yeats**	**Zone of Inhibition diameter in mm**			
			Stems	**Leaves**	**Flowers**	**amphotericin**
1	American type Collection Cultures	*Candida parapsilosis* ATCC220129	11	10	13	25
2	American type Collection Cultures	*Candida albicans* ATCC90028	15	14	17	27
1596	Crachats (From Hospital)	*Candida albicans*	-	-	-	-
1613	Bronchial washing (From Hospital)	*Candida albicans*	11	12	15	21

1628	Intertrigo axillaire (From Hospital)	*Candida albicans*	9	11	12	19
1630	Intertrigo inter-toes (From Hospital)	*Candida albicans*	12	13	14	21
1644	Perionyxis of the inch (From Hospital)	*Candida tropicalis*	7	-	10	17
1672	Vaginal withdrawa (From Hospital)	*Candida albicans*	12	13	14	21
E613	Vaginal withdrawa (From Hospital)	*Candida glabrata*	10	9	-	19
E617	Urines (From Hospital)	*Candida glabrata*	11	11	-	19
1625	Lesion of the back (From Hospital)	*Candida parapsilosis*	8	10	12	20
1627	Perionyxis of the inch (From Hospital)	*Candida parapsilosis*	9	8	7	17
1581	Intertrigo axillaire (From Hospital)	*Candida parapsilosis*	8	8	-	18

-: Not active

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