International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.10 No.3, pp 378-384, 2017

Fatty Acid Composition and Antibacterial Activity of the Leaf Oil of *Kleinhovia hospita* Linn.

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Abstract : The fatty acid composition is utmost important for the quality assessment of leaves. The present study deals with the petroleum ether (60-80°C) extracted oil from the leaves of *Kleinhovia hospita* L. After saponification of leaf oil fatty acids were purified by preparative TLC and the fatty acid composition was determined for the first time by Gas Chromatography (GC) followed by GC-MS techniques after converting the fatty acids into its FAME. Analysis showed that the oil contained nineteen identified fatty acids, accounting 80.47% of the total fatty acids and thirteen unidentified compounds. The predominant fatty acids are palmitic acid (17.97%), linoleic acid (8.05%), oleic acid (7.87%) and stearic acid (7.79%) respectively. Antibacterial activity was also investigated which shows significant values. MICs of the oil against the gram positive (*Bacillus subtilis* and *Bacillus licheniformis*) and gram negative bacteria (*Escherichia coli*and *Acinetobacter junii*) were(61.75µg/ml and 60.02µg/ml) and(35.75µg/ml and 38.04µg/ml) respectively. Based on these results, it can be concluded that the *K. hospita* leaf oil may be applicable in medicine, agriculture and food preservation.
Keywords : *Kleinhovia hospita*, leaf oil, fatty acids, GC-MS, antibacterial activity.

Introduction

Kleinhovia hospita Linn. which is an evergreen, tropical tree growing up to 20 m high native totropical parts of Asia, Africa, and Australia. It is monotypic, being the only species of its genus (family: Sterculiaceae earlier, it is now included in the family Malvaceae)[1]. Various parts of the tree have been used as a folk medicine in parts of Malaya, Indonesia, Papua New Guinea and China[2]. Leaves are eaten as vegetables and the leaf juice is used as eye wash several times due to inflammation in conjunctives relief from pain[3]. The plant is a source of many alkaloids, terpenoids, coumarins and steroids which possesses various biological activities like cytotoxicity, antiproliferative and hepatoprotective and antioxidant activity[2-5].

Humans have used plants as a major source of construction materials, food and medicines for their living system. In the nutrition and health fields, fat is considered as a source of energy in the absence of glucose because it is the most energy-dense nutrient[6]. For this reason, there is a tendency to intake the fats and plant oils as a source of energy. In the food industry, researchers found to search new sources of plant oil that may have nutritional value. The most important factor of different plant oils is the presence of saturated and unsaturated fatty acids in various amounts[7-9].

The present study deals with the chemical characteristics, fatty acid composition and antibacterial activity against gram positive and gram negative bacteria of the *K. hospita* leaf oil.

Materials and methods

Plant materials

Freshmatured leaves of *K. hospita* were collected from the campus area of The University of Burdwan, Burdwan, West Bengal, India. The leaves were collected in the morning during the month of August from the Research Farm (23°53′N latitude and 83°25′E longitude), Department of Botany, The University of Burdwan, West Bengal, India. The plant was authenticated by Prof. Ambarish Mukherjee, Department of Botany, The University of Burdwan, Burdwan, West Bengal, India. Voucher specimens MCD1 for leaves of this plant have been deposited at the herbarium (BURD) of the Department of Botany, The University of Burdwan, Burdwan.

Chemicals

The analytical graded solvents and chemicals used in this experiment were purchased from E. Merck (Mumbai, India). Standard fatty acid methyl esters (FAME) mixture of thirty seven components was purchased from Supelco Chemical Co., (USA).

Oil extraction from the leaves of K. hospita

Fresh and mature leaves of *K. hospita* were dried in air and finely powdered. By the use of soxhlet apparatus, the finely powdered air dried leaves (200 g) were extracted with 2 L petroleum ether (60-80°C) for 72 h. Finally the leaf oil was obtained by the complete removal of the solvent under vacuum. The extracted leaf oil was weighed andstored under nitrogen at 4°C for further analysis. State and color of the oil was noted visually.

Oil content

The weight of oil (per kg) extracted from of dry leaves was determined.

Density

Density of oil was measured by weight of 1 ml of that oil.

Specific gravity

Specific gravity of oil was measured by the ratio of weights of liquid and equal volume of water at 4°C in a specific gravity bottle.

Acid and saponification values

According to the methods of the Association of Official Analytical Chemists acid and saponification values were performed and results are placed in (Table 1)[10].

Infrared spectral analysis

After saponification of the leaf oil infrared spectral analysis (IR) was done in a Shimadzu Prestige-21 FT-IR spectrometer (Japan) for functional group analysis of the mixture of fatty acids. By comparing the library spectral database the functional groups were analysed.

Preparation of FAME

Fatty acid methyl ester (FAME) of the leaf oil was prepared with 12.5% boron trifluoride (BF_3) in methanol[11].Preparative thin layer chromatography (TLC) was performed for purification of FAME using the solvent mixture hexane and ethyl acetate (1:1) as the mobile phase. FAME band was eluted with chloroform. The purified FAME was stored at 4^oC in refrigerator for further analysis.

Gas-Chromatographic analysis of FAME

FAME analysis by capillary gas chromatography (GC) was done on a Shimadzu Gas Chromatograph (Model: GC-2010; Shimadzu, Japan) equipped with a flame-ionization detector (FID) on a split injector. A SP-2560 capillary column (100 m × 0.25 mm i.d.) was used for this analysis. The temperatures of both injection and detector ports were set at 250°C. The oven temperature was 140°C for 5 minutes initially, then raised at 4°C/min to 240°C and finally at 220°C for 20 minutes. The carrier gas nitrogen was a flow rate of 30 ml/min; volume injected 1µl; split ratio, 1:30. By the use of standard FAME obtained from SUPELCO thirty seven Component FAME Mix **(**Sigma-Aldrich Co.) peaks were identified by comparison of their retention times. From the GC peak areas the percentage composition of the samples was computed for the amount of fatty acid present.

GC-MS analysis of the purified FAME

The FAME analysis was done further by Gas Chromatography-Mass Spectrometry on a Shimadzu GCMS- QP 2010 plus (Shimadzu, Japan) fitted with a capillary column [100 m × 0.25 mm i.d]. GC operating conditions were similar as above and the MS condition: ionization voltage 70 eV; ion source temperature, 270°C; and mass range were 30-700 mass units. The individual peaks were identified by comparing their mass spectra with the library mass spectral database.

Antimicrobial assay

Test organisms *Bacillus subtilis* ATCC 6633, *Bacillus licheniformis* ATCC 14580, *Escherichia coli* ATCC 25922 and *Acinetobacter junii* MTCC 11818 were grown on nutrient agar medium (Hi Media). Crude leaf oil of *K. hospita* against test organisms (10^7cfu/ml) having an individual cup diameter of 1 cm by agar diffusion method on nutrient agar medium was done[12]. The inhibition zone of antimicrobial capability was estimated visually. Minimum inhibitory concentrations (MIC) of antibacterial agents against the test organisms were determined by broth dilution methods (EUCAST 2003)[13].

Results and discussion

Oil extraction yield

The leaf oil of *K. hospita*was dirty greenish yellow in color and semi liquid at room temperature (27^{o}C). The yield of chlorophyll containing leaf oil was 28.51g/kg. The physico-chemical properties of the leaf oil were shown in (Table 1). Chemical nature of leaf oil was evaluated by its acid and saponification values.

Table 1. Some physico-chemical properties of the *K. hospita* leaf oil

Parameter	*K. hospita* leaf oil*
Physical state at room temperature	Semi liquid
Total oil content (g/kg)	28.51 ± 0.22
Density (g/ml)	1.33 ± 0.03
Specific gravity	1.30 ± 0.11
Acid value (mg KOH/g)	23.72 ± 0.52
Saponification value (mg KOH)	111.14 ± 0.97

* Values are means ± S.D., n=3

IR spectral analysisof the saponified oil

IR spectrum (Broad band at 3439.08 cm^{-1} for –OH stretching of –COOH group; 2920.2 cm^{-1} and 2850.7 cm^{-1} for $–CH_2$; 1707cm^{-1} C=O str. of –COOH group; 1462.0 cm^{-1} for C=C un-saturation and1379.1cm^{-1} for C-O str. of –COOH group) of the saponified oil indicate the major amount of fatty acids present in the leaf oil (Figure 2).

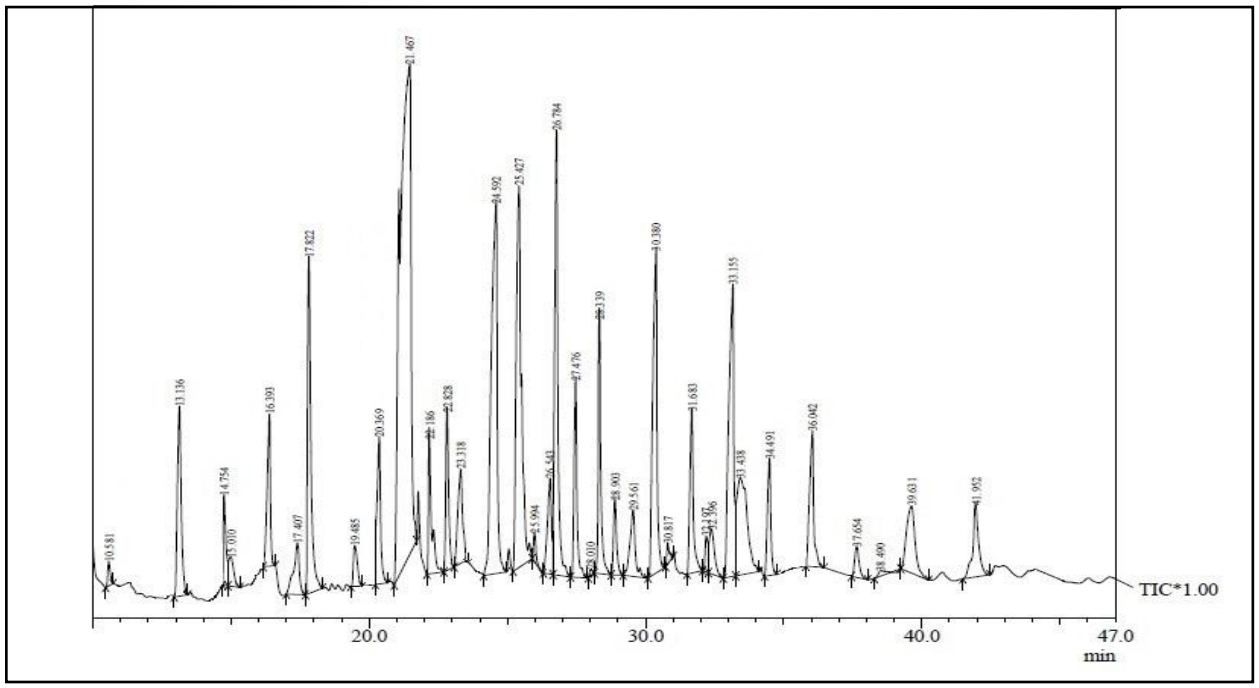

Figure 1. Gas-chromatogram of fatty acid methyl esters of *K. hospita*leaf oil

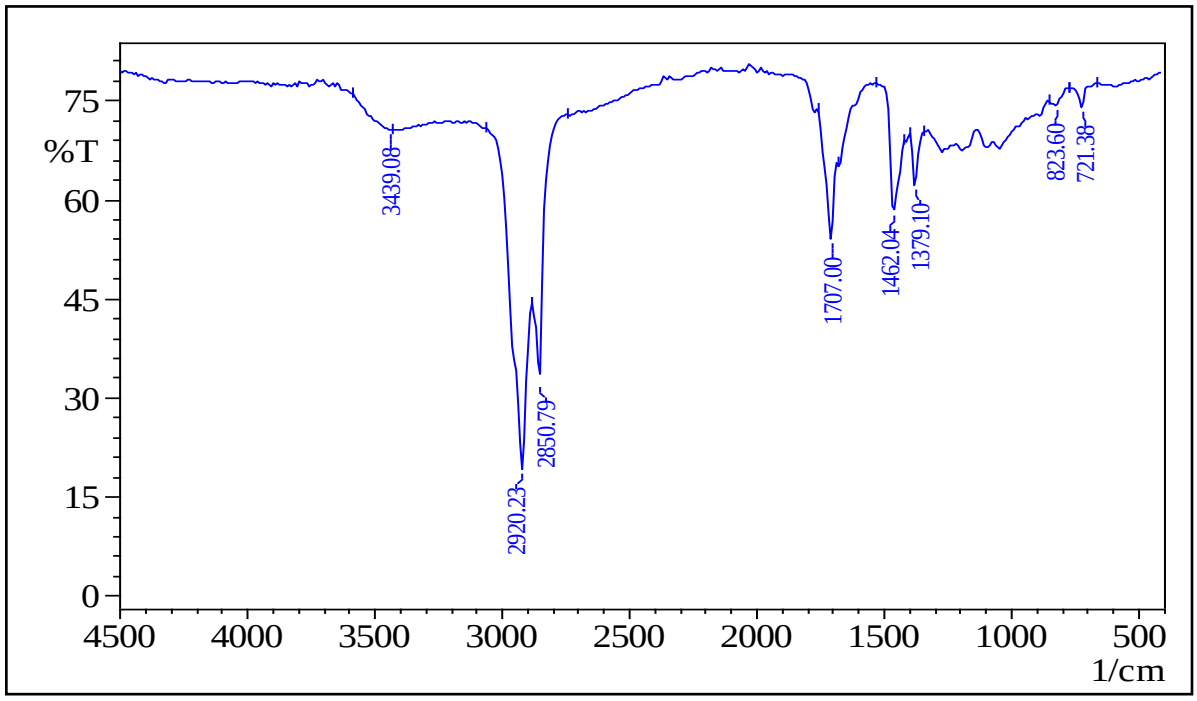

Figure 2. FT-IR of fatty acids of *K. hospita* leaf oil

Fatty acid analysis

The GC-chromatogram was shown in Figure 1 and the fatty acid compositions of the purified FAME mixture were placed in Table 2. From the leaf oil nineteen fatty acids were identified and also quantified (Table 2) that represents 80.47% of the total fatty acids and the rest 19.53% indicated unidentified compounds. The amounts of saturated and unsaturated fatty acids were 58.43% and 22.04% respectively. The most abundant fatty acids were palmitic acid (17.97%), linoleic acid (8.05%), oleic acid (7.87%) and stearic acid (7.79%). Thus, most of the identified fatty acids were present in comparatively good amounts and some of them were present in minute amounts.

Table 2. Fatty acid composition of the *K. hospita* leaf oil

Name of fatty acids	Retention time (min)	Relative percentage*
Unidentified	10.581	0.41 ± 0.02
Unidentified	13.136	2.60 ± 0.07
Lauric acid (C_{12}:0)	14.754	1.12 ± 0.04
Unidentified	15.010	0.69 ± 0.07
Unidentified	16.393	2.05 ± 0.02
Myristic acid (C_{14}:0)	17.822	5.09 ± 0.14
Pentadecylic acid (C_{15}:0)	19.485	0.74 ± 0.00
Unidentified	20.369	1.98 ± 0.10
Palmitic acid (C_{16}:0)	21.467	17.97 ± 0.27
Palmitoleic acid (C_{16}:1)	22.186	1.93 ± 0.06
Margaric acid (C_{17}:0)	22.828	2.32 ± 0.06
Unidentified	23.318	1.71 ± 0.08
Stearic acid (C_{18}:0)	24.592	7.79 ± 0.22
Oleic acid (C_{18}:1)	25.427	7.87 ± 0.19
Nonadecylic acid (C_{19}:0)	25.994	0.66 ± 0.02
Unidentified	26.543	1.62 ± 0.06
Linoleic acid (C_{18}:2)	26.784	8.05 ± 0.17
Arachidic acid (C_{20}:0)	27.476	2.88 ± 0.03
Alpha- linolenic acid (C_{18}:3)	28.339	4.19 ± 0.13
Heneicosylic acid (C_{21}:0)	28.903	1.10 ± 0.07
Unidentified	29.561	1.38 ± 0.02
Behenic acid (C_{22}:0)	30.380	5.19 ± 0.21
Tricosylic acid (C_{23}:0)	31.683	2.51 ± 0.05
Unidentified	32.197	0.62 ± 0.05
Lignoceric acid (C_{24}:0)	33.155	5.00 ± 0.08
Unidentified	33.438	3.64 ± 0.14
Pentacosylic acid (C_{25}:0)	34.491	1.63 ± 0.02
Unidentified	35.503	0.44 ± 0.00
Cerotic acid (C_{26}:0)	36.042	2.35 ± 0.13
Unidentified	37.654	0.69 ± 0.01
Montanic acid (C_{28}:0)	39.631	2.08 ± 0.03
Unidentified	41.952	1.70 ± 0.06

* Values are means ± S.D., n=3

The unsaturated fatty acids: linoleic acid (8.05%) and alpha- linolenic acid (4.19%) are considered as the essential fatty acids and those cannot synthesize in human body but can uptake from foods. Linoleic acid, known as omega-6 fatty acid is one of the naturally occurring essential fatty acid which is found to be beneficial for human health due to its regulation of body fat gain, enhanced immunity and prevention of distinct heart vascular diseases[14, 15]. Another important essential fatty acid, alpha-linolenic acid, is known as omega-3 fatty acid have a beneficial effects in a variety of experimental models of metabolic and chronic inflammatory diseases[16-18]. Thus, presence of both linoleic acid and alpha-linolenic acid in the leaf oil enriches the quality of the oil.

Antibacterial activity

Antibacterial activity of crude oil of leaf extract shows good result against gram positive and gram negative bacteria (Table 3) and it is more significant in case of gram negative bacteria. MIC of fatty acid constituent of saponified leaf oil against two gram positive (*B. subtilis* and *B. licheniformis*) and two gram negative bacteria (*E. coli* and *A. junii*) were determined (Table 4). The exact mechanism of the antibacterial activities of fatty acids is yet to be established. The main target seems to be the bacterial cell membrane and different essential processes like enzyme inhibition, leakage of cell metabolites, celllysis and induction of autolysis, disruption of the electron transport chain, interference with oxidative phosphorylation in it and

inhibition of nutrient uptake. *B. subtilis* and *B. licheniformis* are associated with food spoilage such as ropy bread and incidents of food-borne gastroenteritis. *B. licheniformis* are also associated with septicemia, food poisoning and a common contaminant of dairy products[19].One of the most important bacteria *E. coli* is responsible for enterohaemorrhagic, enterotox-igenic, uropathogenic, meningitis sepsis-associated and watery diarrhoea human infection diseases[20]. *A. Junii* mainly associated with bacteremia in preterm infants and pediatric oncologic patients[21].

Table 3. Antimicrobial activity of *K. hospita* leaf oil against *B. subtilis* and *E. coli*

Antimicrobial substances	**Test organisms**	
	B. subtilis	*E. coli*
	Inhibition zone diameter (cm)	Inhibition zone diameter (cm)
Leaf oil	1.95	1.85
Doxycycline (control)	1.44	1.41

Table 4. MIC of the Fatty acid constituents of *K.hospita* leaf oil against some bacteria

Antibacterial substances	**Test organism MIC (µg/ml)**			
	B. subtilis	*B. licheniformis*	*E. coli*	*A. junii*
Leaf oil	61.75±0.96	60.02±0.82	35.75±0.51	38.04±0.82
Tetracycline (control)	1.50±0.01	2.01±0.02	3.06±0.12	4.66±0.25
Ampicillin (control)	1.0±0.04	3.06±0.12	4.06±0.12	24.25±0.50

* Values are means ± S.D., n=3

Conclusion

Fatty acid composition of *K. hospita* leaf oil shows nineteen identified fatty acids by GC and GC–MS analyses. Saturated fatty acids are present in greater amount than unsaturated ones. Low saponification value, pleasant odour and presence of two essential fatty acids in good amount indicate the stability of the oil from nutritional point of view. The leaf oil also shows antibacterial activity to prevent the growth of bacteria and extend the life of the processed food. The broad spectrum of antibacterial activity enriches the oil for various applications in medicine, agriculture, food preservation and cosmetics.

Acknowledgements

One of the authors (M. C. Dey) is thankful to the University of Burdwan, Burdwan, West Bengal, India, for financial assistance.

References

1. C.S.I.R., New Delhi., The Wealth of India: Raw Materials,1959, 5, 321.
2. Gan, L.S., Ren, G., Mo, J.X., Zhang, X.Y., Yao, W. and Zhou, C.X., Cycloartane triterpenoids from *Kleinhovia hospita*, J. Nat. Prod., 2009, 72(6), 1102-1105.
3. Arung, E.T., Kusuma, I.W., Kim, Y.U., Shimizu, K., and Kondo, R., Antioxidative compounds from leaves of Tahongai (*Klienhovia hospita*), J. Wood. Sci., 2012, 58, 77-80.
4. Arung, E.T., Kusuma, I.W., Purwatiningsih, S., Roh, S.S., Yang, C.H., Jeon, S., Kim, Y.U., Sukaton, E., Susilo, J., Astuti, Y., Wicaksono, B.D., Sandra, F., Shimizu, K. and Kondo, R., Antioxidant activity and cytotoxicity of the traditional Indonesian medicine Tahongai (*Kleinhovia hospita* L.) extract, J. Acupunct. Meridian. Stud., 2009),2(4), 306-308.
5. Zhou, C.X., Zou, L., Ganand, L.S., Cao, Y.L.,Kleinhospitines A–D, New Cycloartane triterpenoid alkaloids from*Kleinhovia hospita*,Org. Lett., 2013, 15 (11),2734-2737.
6. German, J.B. and Dillard, C.J., Composition, structure and absorption of milk lipids: A source of energy, fat-soluble nutrients and bioactive molecules, Crit. Rev. Food Sci. Nutr., 2006, 46(1), 57-92.

7. Basu, S., Kundu, P. and Sinhababu, A., Characterization of fatty acids and sterols composition of seed lipid of *Lagerstroemia speciosa* Pers., Res. Chem. Intermed.,2015,41(9), 6511-6522.
8. Tang, T.F., Liu, X.M., Ling, M., Lai, F., Zhang, L., Zhou, Y.H. and Sun, R.R., Constituents of the essential oil and fatty acid from *Malania oleifera*, Ind. Crops. Prod. 2013, 43, 1-5.
9. Ukil, S., Laskar, S. and Roy, R. N., Physicochemical characterization and antibacterial activity of the leaf oil of *Crotalaria pallida* Aiton., J. Taibah Univ. Sci.,2015, doi.10.1016/j.jtusci.2015.07.001.
10. Association of Official Analytical Chemists, Official methods of analysis. 16th edn. AOAC, Washington, DC, 1995.
11. American Oil Chemists' Society, Official methods and recommended practices of the American Oil Chemists' Socity. 4th edn. Champaign, Method Ce 2, 1992.
12. Washington, J.A., Manual of clinical microbiology, fourth ed. American society for microbiology. Washington, DC, 1985, 967.
13. European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by agar dilution, 2003, 9, 1-7.
14. Pariza, M.W., Perspective on the safety and effectiveness of conjugated linoleic acid, Am. J. Clin. Nutr.,2004, 79, 1132-1136.
15. Calder, P.C., Polyunsaturated fatty acids, inflammation and immunity, Lipids, 2001,36, 1007-1024.
16. Calder, P.C., n-3 polyunsaturated fatty acids, inflammation and inflammatory diseases, Am. J. Clin. Nutr., 2006,83, 1505-1519.
17. Melo, I.L.P., Carvalho, E.B.T. and Mancini-Filho, J., Pomegranate Seed Oil (*Punica Granatum* L.): A source of punicic acid (Conjugated α-linolenic acid), J. Hum. Nutr. Food Sci., 2014, 2(1), 1-11.
18. Li, D., Bode, O., Drummond, H. and Sinclair, A.J., Omega-3 (n-3) fatty acids. In lipids for functional foods and nutraceutricals. ed. by Gunstone, F.D., (The Oily Press, UK), 2003, 225-262.
19. Salkinoja-Salonen, M.S., Vuorio, R., Andersson, M.A., Kampfer, P., Andersson, M.C., Honkanen-Buzalski, T. and Scoging, A.C.,Toxigenic strains of Bacillus licheniformis Related to Food Poisoning. Appl. Environ. Microbiol., 1999, 65(10), 4637-4645.
20. Kaper, J.B., Nataro, J.P. and Mobley, H.L., Pathogenic *Escherichia coli*. Nat. Rev. Microbiol., 2004, 2, 123-140.
21. Kappstein, I., Grundmann, H., Hauer, T. and Niemeyer, C. Aeratorsasa reservoir of *Acinetobacter junii*: an outbreak of bacteraemia in paediatric oncology patients, J. Hosp. Infect., 2000, 44: 27-30.

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