Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Moussa et al., J Plant Pathol Microb 2012, 3:5
DOI: 10.4172/2157-7471.1000132

Research Article Open Access

The Influence of Botanical Origin and Physico-chemical Parameters on the Antifungal Activity of Algerian Honey

Ahmed Moussa[1]*, Djebli Noureddine[2], Aissat Saad[1], Meslem Abdelmalek[1] and Bacha Salima[1]

[1]*Institute of Veterinary Sciences University Ibn-Khaldoun Tiaret, Algeria*
[2]*Department of Biology, Faculty of Sciences, Mostaganem University, Algeria*

Abstract

The purpose of the study was to characterize the physicochemical of 6 natural honeys, and to evaluate the antifungal activity of honey. Honey samples were collected from different locations of Algeria Republic. Pollen profile, moisture content, ash, electrical conductivity and pH, were the parameters analyzed in each honey sample. The antifungal activity of honey samples were tested by 100% and 50% (wt per vol) concentration against *Candida albicans* and *Rhodotorula mucilaginosa,* and by the agar well diffusion method and spectrophotometric assay. Ketoconazole 2% and Nystatin (100 U), were used as positive controls.

The floral identification of honeys allowed to cluster them, as monofloral and polyfloral honeys. Concerning the physicochemical parameters, all honey samples were found to meet European Legislation (EC Directive 2001/110) for all parameters. The mean values obtained for the physico-chemical parameters were: pH 4.1; 15.31% moisture; 0.24% ash, 0.39 ms cm^{-1} electrical conductivity and11.95 free acidity. Inhibition zones for *C. albicans* (6 to10 mm) and *R. mucilaginosa* (6 to 20 mm) were observed. Also, the percentage inhibition(%) for *C. albicans* (69.76 to 99.85) and *R. mucilaginosa* (83.03 to 99.77). The antifungal activity of honeys is related to their floral origin, and physicochemical properties constitute a useful resource for the generation of functional foods.

Keywords: Algerian honey; Botanic origin; Physicochemical parameters; Antifungal activity

Introduction

In recent years, fungal infections, mainly caused by *Candida albicans* and *Rhodotorula mucilaginosa*, are a major cause of morbidity and mortality, and may be life threatening, predominantly in the group of severely immunocompromised patients.

The increased use of antifungal agents also, resulted in the development of resistance to the present drugs. It makes necessary to discover new classes of antifungal compounds, to cure fungal infections. Natural products have been traditionally used in the treatment of diseases, because they are sources of many active compounds. Honey as most natural products, may have a large variance in therapeutic components depending on its origin. Thus, the floral source of honey plays an important role on its biological properties [1].

The composition of honey is rather variable and depends primarily on its floral source; however, certain external factors, such as seasonal and environmental factors and processing, also play a role. The various varieties of honey may be grouped into monofloral or multifloral [2]. The classification basically depends on whether a dominating pollen grain, originated from only one particular plant (monofloral honey) or no dominant pollen type in the sample (multifloral honey) [1,3].

The antimicrobial effect of honey is due to different substances and depends on the botanical origin of honey [4-6]. An extensive review of the antimicrobial activity of honey showed it to be derived from high sugar content, low water content, acidity, the generation of hydrogen peroxide on dilution and phytochemical components [5]. More recently, methylglyoxal was discovered to contribute to the activity of New Zealand's manuka honey [7,8] bee defensin-1 was detected in a Dutch honey [9], and melanoidins were identified in Canadian honeys [6].

In Algeria, thanks to geographical and climatic conditions that provide a suitable environment for apiculture, honey production has been well developed. The beekeeping that has been sustained in Algeria for thousands of years, is an important agricultural activity. Recently, we reported antibacterial and antifungal properties of Algerian honeys [10-14]. The aim of this work was to evaluate the physicochemical parameters and antifungal activity characteristics of honey of different botanical sources, from four geographic origin of Algeria.

Materials and Methods

Honey samples and their Preparation

A total number of 6 honey samples were obtained from beekeepers in four regions of western Algerian: Saida (n=1); Tiaret (n=3); Relizane (n=1); Mascara (n=1) (Table 1). All samples were collected in their original packages and were transferred to the laboratory, and kept at 4-5°C, until analysis.

Pollen analysis

The preparation of honey samples for identification of botanical origin, followed the standardized method of Erdtman G [15]. Pollen identification was based on the reference collection from the LABORATOIRE DU CARI LOUVAIN-LA-NEUVE (Belgique).

***Corresponding author:** Ahmed Moussa, Institute of Veterinary Sciences University Ibn-Khaldoun Tiaret (14000), Algeria, Tel: +213 65234059; Fax: +213 46 425001; E-mail: moussa7014@yahoo.fr

Received August 12, 2012; **Accepted** September 19, 2012; **Published** September 24, 2012

Citation: Ahmed M, Djebli N, Aissat S , Meslem A, Bacha S (2012) The Influence of Botanical Origin and Physico-chemical Parameters on the Antifungal Activity of Algerian Honey. J Plant Pathol Microb 3:132. doi:10.4172/2157-7471.1000132

Sample	Description	Collection	
		Season	Place of origin
H1	Unifloral	2011	Saida
H2	Multifloral	2011	Mascara
H3	Multifloral	2011	Tiaret
H4	Unifloral	2011	Tiaret
H5	Unifloral	2011	Relizane
H6	Multifloral	2011	Tiaret

Table1: Collection location, description and time of collection, of natural honey samples from Algeria.

Physicochemical analyses

All physicochemical tests were performed in triplicate: Water content (moisture) was determined by an Abbe-type refractometer reading at 20°C, according to the relationship between honey water content and refractive index [6,16].

Measurements of pH were performed with a pH meter ((Hanna Instruments), in a solution containing 10 g of honey in 75 ml of CO_2 free distilled water.

Honey electrical conductivity was measured at 20°C [17] with a Crison Basic 30 conductimeter. Results were expressed in microSiemens per centimeter (µS/cm) [9,18].

Ash content

Total ash was estimated by conductimetry, using the equation:

Ash content (%) = 0,083- conductivity- 0.092 [19]

Free acidity

Free acidity was determined by potentiometric titration [17]. Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. Ten grams of honey were then dissolved in 75 mL of distilled water, and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined, as 10 times the volume of NaOH used in titration.

Antifungal activity

Honey solutions were prepared in two concentrations: 100% and 50% (wt/vol). The samples of each honey (10 g) and sterile water were stored at 37°C for 30 min before mixing, to facilitate homogenization. The potential antifungal activity of 6 selected natural honeys, against two species of fungi was studied, using the spectrophotometric assay and agar well diffusion method.

Culture media and inoculum

Candida albicans ATCC 10231 and *Rhodotorula mucilaginosa* (clinical isolate), were grown on Sabouraud Dextrose Agar (SDA; Merck, Germany), for 24 h at 37°C. Yeast cells from at least five colonies (1 mm diameter) were suspended in 5 mL of sterile saline solution (0.85%), and the resulting yeast suspension was mixed for 15 s in a vortex. Then, the suspensions were adjusted by spectrophotometric method, adding saline solution, to reach the value of 0.5 in the McFarland scale corresponding to a final concentration of $3.0 \pm 2.0 \times 10^6$ cells/ mL.

Agar well diffusion method

The agar well diffusion method was employed. The honey samples were first inoculated separately on standard nutrient media with no test organisms, so as to evaluate their possible contamination. Thereafter, solidified SDA agar plates (90 mm diameter) were separately flooded with the liquid inoculums of the different test organisms, using the spread plate method. The plates were drained and allowed to dry at 37°C for 30 min, after which four equidistant wells of 8mm in diameter were punched, using a sterile cork borer at different sites on the plates. 50 µl of the different concentrations, 50% (wt/vol), and undiluted of the honey samples were separately placed in the different punched wells with 1 ml sterile syringe. The plates were allowed to stay for 15 min for pre-diffusion to take place, followed by an overnight incubation that lasted for 28 h at 37°C. The diameter of inhibition zones, including the diameter of the well, was recorded. The experiment was repeated twice.

Spectrophotometric assay

Up to 0.2 ml of the cell suspension was inoculated into 4 ml volume of honey concentration in a test tube, while inoculation of 4 ml volume of nutrient broth with 0.2 ml of the cell suspension, served as control. The optical density was determined in a spectrophotometer at 620 nm, prior to incubation (T_0) and recorded after which, the cultures were incubated for 24 hours in the dark at 37°C with constant shaking, to prevent adherence and clumping. After 24 hours of incubation, the optical densities were again determined (T_{24}) and recorded. The optical density for each replicate at T_0 was subtracted from the optical density for each replicate at T_{24}. The growth inhibition for the test at each dilution was determined, using the formula: Percentage inhibition = 1 - (OD test/OD control) x 100.

Where the resulting measurement recorded a negative inhibition value (growth promotion), this was reported as stimulation using the formula: Percentage inhibition = (OD test/OD control) x 100. The minimum and maximum values were 0% and 100%.

Results and Discussion

Results of honeys pollen profile analysis permits to determinate its floral origin. The identified pollens and its frequency on the six analyzed honeys are presented on Table 1. The most relevant differences among the five honeys were the type and amount of the most predominant pollen present in them.

The percentages of the most abundant pollen types in each honey sample, as well as the nectar and pollen character of these plants were taken into account. Following the criteria of Zander [20], when the percentage of the most abundant pollen type was over 45%, the honey sample was classified as unifloral, with an exception of eucalyptus pollen at 70%. Lower percentages were classified as polyfloral (Table 2).

Physicochemical parameters

The six honey samples were studied, in terms of chemical properties, and tests were performed in triplicate. Moisture is a parameter related to the maturity degree of honey and temperature. In the present study, moisture values are between 13.76% and 16.30% (Table 3). One sample with 20.2% exceeded the limit (20%) allowed by European Community regulations, The Council of the European Union [21]. Moisture values were within the values found in Marocco honeys (between 14.64% and 18.59%) Chakir et al. [22] and less than those found in Moroccan honeys (between 14.5% and 23.6%) Terrab et al. [23]. The moisture content of honey depends on various factors such as harvesting season, degree of maturity reached in the hive and climatic factors Finola et al. [24]. Also, the moisture content of honey is highly important factor contributing to its stability against fermentation and granulation during storage [25].

All the Algerian honeys analysed were found to be acidic in character. Their pH values ranged from 4 to 4.6 (Table 3). In general,

Sample/Colour	Frequency class[a]	Pollen identification (frequency)
Honey 1	A* S I	Apiacées **(48%)** Ronces **(15%)**, Brassicacées **(16%)** Renoncule, Astéracées, Lamiacées, Trèfle, Cistacée, Rosacées, **Fabacée**
Honey 2	S I	Rosacées **(13%), Fabacée (23%),** Apiacées **(24%)** Chénopodiacées, Convolvulacées, Lamiacées, Malvacées, Plantain, Poacées, Viperines, Eucalyptus, Renoncule, Cistacée, Rhamacées, Ronces , Brassicacées,Astéracées
Honey 3	A* S I	Rhamnacées **(47%)** Astéracées (17**%),** Eucalyptus **(18%)** Lamiacées, Lotier, Vesce, Ronces , Brassicacées, Rosacées, Apiacées, **Fabacée**
Honey 4	A* I	Eucalyptus (87%) Plantain, Renoncule, Viperine, Apiacées, Brassicacées, Cistacée, Citrus, Ronces , Fabacée, Astéracées, Rosacées
Honey 5	S I	Astéracées **(13%),** Apiacées **(23%),** Brassicacées **(25%),** Oranger **(27%)** Acacia, Rosacées, Cistacée, Lamiacées, Renoncule, Ronces, **Fabacée,** Eucalyptus
Honey 6	A* S I	Astéracées **(62%)** Rosacées **(11%),** Brassicacées **(16%)** Lamiacées, Vesce, Apiacées, Chénopodiacées, Eucalyptus, Fabacée, Ronces

Table 2: [a]Frequency classes: P – predominant pollen (more than 45% of pollen grains counted); S – secondary pollen (10–40%); I – important minor pollen (< 10%).

Physico-chemical analysis	H1	H2	H3	H4	H5	H6
Moisture %	16.04 ± 0.2	13.76 ± 0.2	14.87 ± 0.2	14.68 ± 0.2	16.3 ± 0.2	16.24 ± 0.2
PH	4.1 ± 0.2	4.1 ± 0.2	4.6 ± 0.2	4 ± 0.2	4 ± 0.2	4.2 ± 0.2
Free acidity (meq Ac/kg)	17.3 ± 1.05	15.2 ± 1.05	8.3 ± 1.05	11.3 ± 1.05	9.9 ± 1.05	9.7 ± 1.05
Electrical conductivity (mS/cm)	0.52 ± 0.01	0.47 ± 0.01	0.51 ± 0.01	0.38 ± 0.01	0.2 ± 0.01	0.28 ± 0.01
Ash %	0.35 ± 0.01	0.31 ± 0.01	0.34 ± 0.01	0.23 ± 0.01	0.08 ± 0.01	0.15 ± 0.01

Table 3: Results of physic-chemical parameters of 6 Algerian honey samples (mean ± standard deviation, n = 3).

honey is acidic in nature, irrespective of its variable geographical origin. The pH values of Indian, Morocco, Argentinean honeys and Saudi honeys, have been found to vary between 3.7 to 4.4, 3.91 to 4.93, 3.25 to 3.32 and 3.48 to 6.06, respectively [26-28]. This parameter is of great importance, during extraction and storage of honey, as it influences the texture, stability and shelf life of honey [29].

Ash content is one of these parameters that have been associated with botanical and geographical origins of honey samples. The ash content in honey is generally small, and depends on nectar composition of predominant plants in their formation [8].

Its value in the analysed samples, ranged from 0.08 to 0.35 g% (Table 3). These results are good agreement with those of Al-Khalifa et al. [30], Sahinler and Gul [31] and Sudhanshu S et al. [32].

The electrical conductivity values of honey samples, tested in our study are listed in (Table 3).

The electrical conductivity (mS/cm) in honey samples, varied in the range of 0.20- 0.52. A linear relationship is known to exist between the ash content and the electrical conductivity, which is expressed as C = 0.14 + 1.74 A, where C is the electrical conductivity and A is the ash content Bogdanov et al. [33]. The coefficient of correlation between electrical conductivity and ash was found to be 0.98, which indicated a strong positive correlation between the two parameters. Similarly, a correlation value of 0.92 has been found to exist between the electrical conductivity and ash content, for some Algerian honeys [28]. The electrical conductivity of honey is closely related to the concentration of mineral salts, organic acids and proteins. This parameter shows great variability according to the floral origin, and is important for differentiating honeys of different floral origins.

The values of acidity obtained ranged from 8.3 meq/ kg in honey harvested, using modern method to 17.3 meq/kg (Table 3) in honey harvested using traditional method.

All honeys presented free acidity values, below 40 meq NaOH kg^{-1}. Free acidity may be explained by taking into account the presence of organic acids, which are proportional to the corresponding lactones, or internal esters, and some inorganic ions such as phosphate or sulphate [24]. Makhloufi et al. reported total acidity of honey from different regions of Algeria, to range from 3.0 to 22.5 meq/ kg, and this indicated the absence of undesirable fermentation. Sahinler and Gul [31] also reported varying acidity values for honey produced from sunflower, cotton, orange and pine to be 40.73, 25.24, 34.96 and 25.76 meq/ kg, respectively.

Inhibitory activity evaluation

The antifungal activity of the 6 honey samples was first measured by agar diffusion, which is suitable for a previous screening test.

The growth inhibition profiles of six honeys are presented in figure 1 and 2. A variation in the antifungal activity with floral source was observed.

These results are in accordance with those reported in other studies, which showed that the response of the different bacterial species to the treatment with a certain honey sample, is rather dependent on the type of honey and the individual organism within a category.

Fungal infection is becoming a serious medical problem, because of the difficulty of its control in immunocompromised individuals, and because of the emergence of multidrug-resistant fungi, although a variety of antifungal drugs have been developed [34,35]. *Rhodotorula species*, including *R. mucilaginosa* (*R rubra*) and *R. glutinis,* are often resistant to fluconazole and voriconazole [36]. The treatment of invasive Candida infections is often complicated by high toxicity, low tolerability or a narrow spectrum of activity of the current antifungal

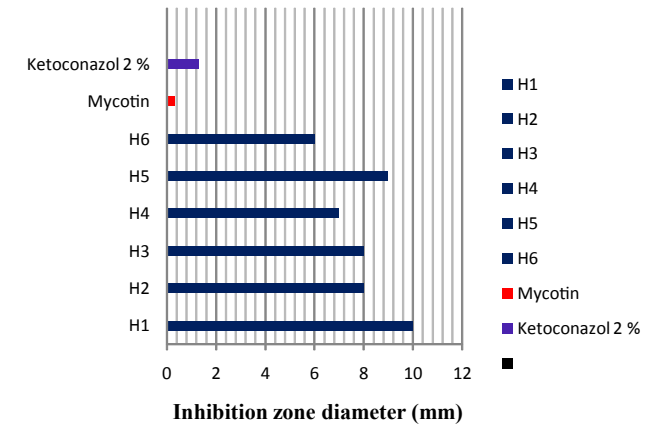

Figure 1: Antifungal activity of tested products against *C. albicans* ATCC 10231.

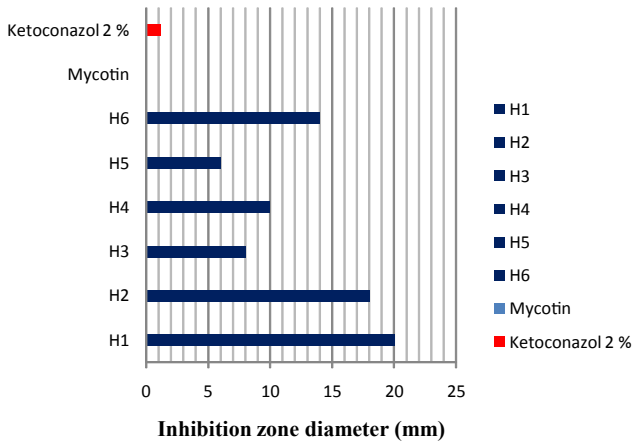

Figure 2: Antifungal activity of tested products against *R. mucilaginosa*.

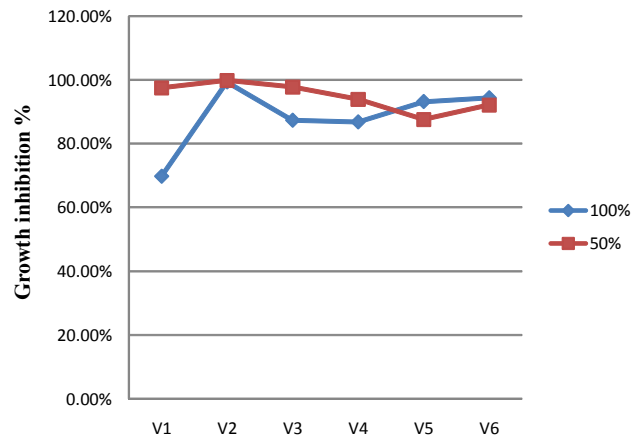

Figure 3: Relationship between the level of antifungal activity against *C. albicans* and honey floral origin.

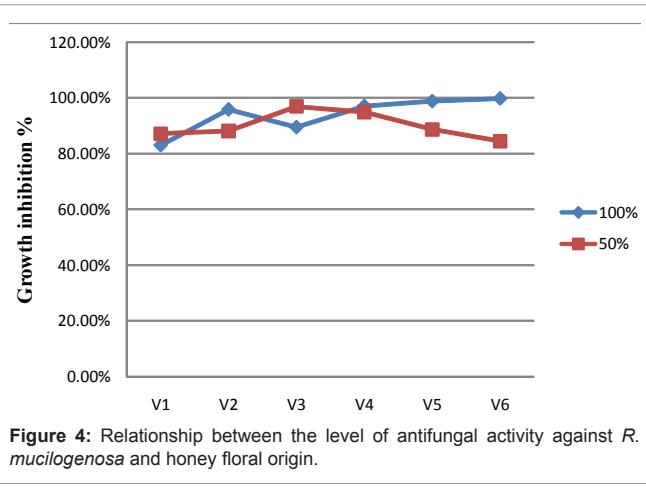

Figure 4: Relationship between the level of antifungal activity against *R. mucilogenosa* and honey floral origin.

drugs, as well as an increase in the incidence of azole-resistant strains [37]. Therefore, the search for new antifungal agents is necessary and stimulates research on new chemotherapeutic agents in natural products. Different natural substances are responsible for antifungal action. Honey is a natural product that is used for its antifungal activity [14]. A number of studies have reported the antifungal properties of different types of honey. For example, Koc et al. [38] reported antifungal efficacy of various honeys, against clinical isolates of *Candida albicans, C. krusei, C. glabrata* and *Trichosoporon spp* (Figure 3). Moussa et al. [12] found that honey concentration ranging from 50% to 100%, inhibited the growth of several pathogenic microorganisms, including *C. albicans* and *Rhodotorula sp*. Nevertheless, there is no information on antifungal activity of Algeria honey against *Rhodotorula mucilaginosa* in the literature (Figure 4). Additionally Obaseiki-Ebor et al. [39] demonstrated the susceptibility of 72 isolates of *C. albicans*, to honey. Khosravi et al. [40] reported that honey had antifungal activity against *Candida species* such as *Candida albicans*, *C. parapsilosis*, *C. tropicalis, Candida kefyr, C. glabrata,* and *C. dubliniensis.*

Previous reports demonstrated that increased honey concentrations resulted in reduced growth of *C. albicans*, namely 29.4% inhibition on the growth was verified, in the presence of wasbessie honey at concentrations of 25% [41].

Several factors may influence the antifungal activity of honey. For example, DeMera and Angert [42] reported that honey from different phytogeographic regions, varied in their ability to inhibit the growth of yeasts, suggesting that botanical origin plays an important role in influencing the antifungal activity. In addition, there are a great variety of components, including phenolic acids, flavonoids and other biomolecules, in different honeys.

The variation in the antifungal potential of honey samples used in this study, as compared to the previous similar studies, highlights that the source of the nectars may have contributed to the difference in the antifungal activities of honey, i.e. the flowers from which bees gathered nectar to produce the honey, since flora source determines many of the attributes of honey, for example, flavor, aroma, color and composition.

References

1. Molan PC (2002) Re-introducing honey in the management of wounds and ulcers: theory and practice. Ostomy Wound Manage 48: 28-40.
2. Molan PC (1998) The limitations of the methods of identifying the floral source of honeys. Bee World 97: 59-68.
3. Ramírez-Arriaga E, Navarro-Calvo LA, Díaz-Carbajal E (2011) Botanical characterisation of Mexican honeys from a subtropical region (Oaxaca) based on pollen analysis. Grana 50: 40-54.
4. Molan PC (1997) Honey as an antimicrobial agent. Bee Products: Properties, Applications and Apitherapy. Plenum Press, New York.
5. Molan PC (1992) The antibacterial activity of honey 1. The nature of the antibacterial activity. Bee World 73: 5-28.
6. Bogdanov S (2002) Harmonized Methods of the European Honey Commission. International Honey Commission.
7. Mavric E, Wittmann S, Barth G, Henle T (2008) Identification and quantification of methylglyoxal as the dominant antibacterial constituent of Manuka (Leptospermum scoparium) honeys from New Zealand. Mol Nutr Food Res 52: 483-489.
8. Adams CJ, Boult CH, Deadman BJ, Farr JM, Grainger MN, et al. (2008) Isolation by HPLC and characterization of the bioactive fraction of New Zealand manuka (Leptospermum scoparium) honey. Carbohydr Res 343: 651-659.
9. Krauze A, Zalewski RI (1991) Classification of honeys by principal component analysis on the basis of chemical and physical parameters. Chemistry and Materials Science 192: 19-23.
10. Ahmed M, Aissat S, Djebli N, Boulkaboul A, Abdelmalek M, et al. (2011) The Influence of Starch of Ginger on the Antibacterial Activity of Honey of Different Types from Algeria against Escherichia coli and Staphylococcus aureus. IJMR 2: 258-262.
11. Ahmed M, Djebli N, Hammoudi SM, Aissat S, Akila B, et al. (2012) Additive

potential of ginger starch on antifungal potency of honey against *Candida albicans*. Asian Pac J Trop Biomed 2: 253-255.

12. Ahmed M, Djebli N, Aissat S, Meslem A , Benhalima A (2012) Antifungal activity of four honeys of different types from algeria against pathogenic yeast: *Candida albicans* and *Rhodotorula sp*. Asian Pac J Trop Biomed 2: 554-557.
13. Ahmed M, Djebli N, Meslem A, Saad A (2012) Antibacterial activity of various honey types of Algeria against Pathogenic Gram-Negative Bacilli: *Escherichia coli* and *Pseudomonas aeruginosa*. Asian Pacific Journal of Tropical Disease 2: 211-214.
14. Ahmed M, Djebli N, Saad A, Meslem A (2012) Anti-Aspergillus niger of eucalyptus honey influenced by thermal treatment. Scientificreports1: 2.
15. Erdtman G (1969) Handbook of Palynology. Munksgaard, Copenhagen.
16. Chataway HD (1932) Determination of moisture in honey. Canadian Journal of Research 6: 532-547.
17. AOAC (1990) Official methods of analysis. (15thedn), Association of official Analytical Chemists, Inc Arlington, VA, USA.
18. Mateo R, Bosch-Reig F (1997) Sugar profiles of Spanish unifloral honeys. Food Chem 60: 33-41.
19. Sancho MT, Muniategui S, Huidobro JF, Simal J (1992) Evaluating soluble and insoluble ash, alkalinity of soluble and insoluble ash and total alkalinity of ash in honey using electrical conductivity measurements at 20°C. Apidologie 23: 291-297.
20. Zander E (1950) The service palynology study of honey. Annals Spanish Institute Soil Science 9: 195-209.
21. The Council of the European Union (2002) Council Directive 2001/ 110/ EC of 20 December 2001 relating to honey. Official Journal of the European Communities 1: 47-52.
22. Chakir A, Abderrahmane R, Gian LM, Paola F (2011) Physicochemical properties of some honeys produced from different plants in Morocco. Arabian Journal of Chemistry.
23. Terrab A, Gustavo González A, Díez MJ, Heredia FJ (2003) Characterisation of Moroccan unifloral honeysusing multivariate analysis. Eur Food Res Technol 218: 88-95.
24. Finola MS, Lasagno MC, Marioli JM (2007) Microbiological and chemical characterization of honeys from central Argentina. Food Chemistry 100: 1649-1653.
25. Singh N, Bath PK (1997) Quality evaluation of different types of Indian honey. Food Chem 58: 129-133.
26. Azeredo LDC, Azeredo M, De Souza SR, Dutra VML (2003) Protein content and physicochemical properties in honey samples of Apis Mellifera of different floral origins. Food Chemistry 80: 249-254.
27. Kayacier A, Karaman S (2008) Rheological and some physicochemical characteristics of selected Turkish honeys. Journal of Texture Studies 39: 17-27.
28. Ouchemoukh S, Louaileche H, Schweizer P (2007) Physicochemical characteristics and pollen spectrum of some Algerian honey. Food Control 18: 52-58.
29. Terrab A, Recamales AF, Hernanz D, Heredia FJ (2004) Characterisation of Spanish thyme honeys by their physicochemical characteristics and mineral contents. Food Chem 88: 537-542.
30. Al-Khalifa AS, Al-Arify IA (1999) Physicochemical characteristics and pollen spectrum of some Saudi honeys. Food Chemistry 67: 21-25.
31. Sahinler N, Gul A (2004) Highland and biochemical analysis of sunflower honey i. 4. National Animal Science Congress, Isparta, Turkey.
32. Sudhanshu S, Satyendra G, Arun S (2010) Physical, biochemical and antioxidant properties of some Indian honeys. Food Chem 118: 391-397.
33. Bogdanov S (2010) Physical Properties of Honey. Bee Product Science.
34. Sullivan DJ, Moran GP, Pinjon E, Al-Mosaid A, Stokes C, et al. (2004) Comparison of the epidemiology, drug resistance mechanisms, and virulence of Candida dubliniensis and Candida albicans. FEMS Yeast Res 4: 369-376.
35. Cornet M, Fleury L, Maslo C, Bernard JF, Brücker G, et al. (2002) Epidemiology of invasive aspergillosis in France: a six-year multicentric survey in the Greater Paris area. J Hosp Infect 51: 288-296.
36. Pfaller MA, Diekema DJ, Gibbs DL, Newell VA, Meis JF, et al. (2007) Results from the ARTEMIS DISK global antifungal surveillance study, 1997 to 2005: an 8.5-year analysis of susceptibilities of Candida species and other yeast species to fl uconazole and voriconazole determined by CLSI standardized disk diffusion testing. J Clin Microbiol 45: 1735-1745.
37. Canuto MM, Rodero FG (2002) Antifungal drug resistance to azoles and polyenes. Lancet Infect Dis 2: 550-563.
38. Koc AN, Silici S, Kasap F, Hörmet-Oz HT, Mavus-Buldu H (2008) Antifungal activity of Turkish honey against Candida spp: and Trichosporon spp an in vitro evaluation. Med Mycol 47: 707-712.
39. Obaseiki-Ebor EE, Afonya TC (1984) *In-vitro* evaluation of the anticandiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents. J Pharm Pharmacol 36: 283-284.
40. Rhosravi A, Shokri H, Katiraee F, Ziglari T, Forsi M (2008) Fungicidal potential of different Iranian honeys against some pathogenic Candida species. J Apicult Res 47: 256-260.
41. Theunissen F, Grobler S, Gedalia I (2001) The antifungal action of three South African honeys on Candida albicans. Apidologie 32: 371-379.
42. DeMera JH, Angert ER (2004) Comparison of the antimicrobial activity of honey produced by Tetragonisca angustula (Meliponinae) and Apis mellifera from different phytogeographic regions of Costa Rica. Apidologie 35: 411-417.