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Research Article

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Evaluation of antioxidant, antibacterial and antifungal activities of eleven monofloral honey samples collected from Morocco

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ABSTRACT

Eleven honey samples collected from Morocco were examined for their relative phenolic and flavonoid contents as well as antioxidant, antibacterial, and antifungal activities. Total phenolic and flavonoid contents were determined using colorimetric assays. The antioxidant ability was assessed by measuring reducing power, scavenging of ABTS and DPPH radicals and β-carotene bleaching assay while antibacterial and antifungal activities were evaluated by disc diffusion and minimum inhibitory concentration assays against a set of fungal and bacterial strains.Among tested honey samples, Thym honey was found to exhibit a highestphenolic (1138.53 mg GAE/kg), flavonoid (179.08 mg RE/kg) content and showed potent antioxidant activity based on ABTS (2.73 mmol TE/kg), DPPH (IC_{50}=5.52 mg/ml), β-carotene bleaching assay (IC_{50} = 24.32 mg/ml). While Oranger honey contains the lowest phenolic and flavonoid content and revealed the lowest antioxidant activity. Concerning the antimicrobial activity and based on minimum inhibitory concentration assay, Thym honey was found to be the most effective growth inhibitor against B. subtilis (6.00%), S. pyogenes (7.33%), E. coli (7.66%), P. aeruginosa (9.50%),C. neoformans (15.16%), and R. mucilaginosa (16.83%).While Acacia honey was found to be most effective against S. aureus (5.16%), S. abony (7.83%) and C. albicans (21.33%). Orange honey was the least effective against all bacteria and fungi strains. The results of this investigation suggest that honey could be considered as a good source of new antimicrobial and antioxidant agents that might prevent several diseases.

Keywords: honey, phenolic, antioxidant, antibacterial, antifungal

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INTRODUCTION

Honey is a natural sweet substance made by bees using nectar or honeydew. This natural product has been widely accepted, as a nutraceutical agent; by all generations, traditions, and civilizations, both ancient and modern; thanks to their nutritional benefits and therapeutic promises.

Honey has been used in Indian folk medicine to keep the teeth and gums healthy, as boon to those with weak digestion and to treat irritating cough, insomnia, skin disorders (such as wounds and burns), imbalances of the lungs and anemia, cardiac pain and palpitation, eye ailments (such as cataract) [1]. In Greek medical system, honey has been used to treat gout and certain nervous disorders, baldness, acute fevers, contraception, eye diseases, cough and sore throat and scars [2]. Avicenna the great Islamic and Iranian physician had recommended honey as one of the best remedies in the treatment of tuberculosis [3].

Laboratory and clinical investigations conducted on honey have been reported to have antimicrobial, antiviral, antifungal, anti-inflammatory, antioxidant, anticarcinogenic activities, the oral administration of honey protects against cardiovascular and gastrointestinal tract diseases as showed several researches[1].

Honey contains a wide range of phytochemical compounds beside fructose and glucose, which are the predominant compounds. Honey contains vitamins, minerals, amino acids, Millard reaction products, peptides, flavonoids (such as kaempferol, apigenin, quercetin, galangin, pinocembrin, chrysin), phenolic acids (such as caffeic, ellagic, ferulic and p-coumaric acids), tocopherols, ascorbic acid, reduced glutathione (GSH), enzymes such as superoxide dismutase (SOD) and catalase (CAT) [4,5]. These compounds may work together to provide a synergistic biological activity observed for honey.

The aim of this study is to investigate the antioxidant, antibacterial and antifungal activities of eleven honey samples produced in Morocco.

EXPERIMENTAL SECTION

1.1. Antioxidantactivity

1.1.1.Measurement of Total phenolic content:

The total phenolic contents in honey samples were determined according to the method described byBouhlali et al[6]. Briefly, 100 µL of the honey dilution were added to 500 µL of a 1/10 dilution of Folin–Ciocalteau reagent in the water, then400µL sodium carbonate solution (7.5% w/v) was added. The mixture was left for 60 min at room temperature and the absorbance was measured at 765 nm.The calibration curve was prepared using Gallic acid. The total phenolic compounds were expressed as mg of Gallic acid equivalent (GAE) /kg of honey.

1.1.2.Measurement of flavonoid content:

The total flavonoid content of honey samples was determined by the method ofKim et al[7]. One mL of honey dilution was mixed with 4mL of distilled water. Then 0.3 mL of sodium nitrite solution (5%) was added, followed by 0.3 mL of aluminum chloride solution (10%). Test tubes were incubated for 5 min at ambient temperature, and then 2 mL of sodium hydroxide (1M) were added to the mixture, then the final volume was made up to 10 mL with distilled water. The mixture was thoroughly vortexed and the absorbance was determined at 510nm. Measurements were calibrated to a standard curve of the prepared Rutin solution and the results were expressed as mg Rutin equivalents (RE) /kgof honey.

1.1.3.Ferric reducing antioxidant power assay:

The ferric reducing activity of honey samples was estimated based on the method of Benzie et al[8]. The FRAP reagent was prepared by mixing 50 mL of acetate buffer (0.3 M at pH 3.6), 5mL of TPTZ (2,4,6-Tripyridyl-s-Triazine) solution (10mM prepared in HCl (40 mM)) and 5mL of Ferric chloride solution ($FeCl_3$) (20mM). 2 mL of the freshly prepared FRAP reagent were added to 10µL of diluted honey. The absorbance was measured at 593 nm against the blank after 10 minutes at room temperature. The standard curve was constructed using Trolox. The result was expressed as Trolox equivalent in µmol/kg of honey.

1.1.4.DPPH radical scavenging activity:

Radical scavenging activity of plant material against stable DPPH was evaluated using the method describedbyBouhlali et al[9]. The reaction mixture contained 100 µL of diluted honey samples at different concentrations and 1.9 mL of methanolic DPPH (0.3mM). The result mixtures were left at room temperature for 20 min and the absorbance was measured at 517 nm. The IC_{50} (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of honey samples. Trolox a stable antioxidant, was used as a synthetic reference.

$$\%\ inhibition\ = \frac{\left(\text{Abs (control)} - \text{Abs (sample)}\right)}{\text{Abs (control)}} X100$$

Abs control is the absorbance without extract; Abs sample is the absorbance of the extract or standard.

1.1.5.*β-Carotene bleaching assay***:**

The β-carotene bleaching inhibition method was carried out using the method of Shahidi et al[10]. Two mg of β-carotene were dissolved in 10 mL of chloroform then 4 mL of this solution were pipetted into a round-bottom flask, which contains 40 mg of linoleic acid and 500 mg of Tween 40. The chloroform was then, evaporated under vacuum at 40°C and 100 mL of oxygenated water was added and vigorously shaken to yield fresh an emulsion. 1 mL of the emulsion was transferred into test tubes containing 100 µL of diluted honey samplesat different concentration and incubated in a water bath at 50°C then the absorbance was measured at 470 nm immediately (t = 0 min) and after 120 min of incubation against a blank which contains the emulsion without β-carotene.The antioxidant activity of honey samples was compared to the positive control, which was Trolox in this assay. The β-carotene bleaching inhibition (%) of the analyzed solution was calculated via the following formula:

$$\beta \text{ carotene bleaching inhibition } (\%) = \frac{\beta \text{ carotene content after 2 h assay}}{\text{Initial } \beta \text{ carotene content}} \text{x } 100$$

The IC_{50} was calculated using a standard curve between the plant material concentration and the percentage of β-carotene bleaching inhibition.

1.1.6.ABTS radical scavenging assay:
The ABTS radical scavenging was measured using the method of Re et al[11]. The ABTS radical cations (ABTS+) were produced by reacting aqueous solution of ABTS (7mM) with an aqueous solution of potassium persulphate (2.45mM). The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use, then diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. 30µL of diluted honey added to 3 mL of the ABTS radical solution were allowed at room temperature for 6 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained using aqueous solution of Trolox. The total antioxidants were expressed as µmol of Trolox equivalents (TE) per kg of honey.

1.2. Antibacterial and antifungal activities:
1.2.1.Microbial strains:
Six bacteria strain includes three Gram-positive: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Streptococcus pyogenes*ATCC 19615 and three *Gram negative*: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella abony* NCTC 6017 were used in the determination of antibacterial activity.

Candida albicans ATCC 18804, *Cryptococcus neoformans*ATCC 32608 and *Rhodoturula mucilaginosa*Y0478 were used in the determination of antifungal activity.

These Microorganisms were obtained from the culture collection of The National Institute of Hygiene (Rabat).

1.2.2.Disc-diffusion method:
The disc-diffusion test of honey samples was carried out using the method described by Chandrasekaran et al[12]with slight modification. Briefly 250 µL of inocula (turbidity of a 0.5 McFarland standard), prepared in physiological saline using twenty-four hours old culture of selected bacteria/yeast were spread over the plates containing Mueller Hinton Agar for bacteria and Sabouraud Dextrose Agar for fungi. The paper disc impregnated with 15 µl of differentdiluted honeysampleswas placed on the surface of the media. Then the diameter of an inhibition zone was measured around the discs after incubation of Petri dishes at 37°C for 24 h (bacteria), 28°C for 48 h (yeasts). One other Petri dish was used as a negative control contains the bacterial cell suspension without the test extract. This assay was done in triplicates, and the inhibition zones of honeys were compared with those of gentamicin (10µg/disc) for bacterial strain.

1.2.3.Minimum Inhibitory Concentration (MIC):
The antimicrobial activity of the various honeys was tested using the micro-dilution method of Bouhlali et al[13]. First of all, the resazurin solution was prepared by dissolving one tablet in 40 mL of sterile water. The bacterial and fungal cultures were prepared by diluting an overnight culture (24h at 37°C) of a test strain in 0.1% physiologic water to match the turbidity of a 0.5 McFarland standard. Afterwards 100 µL of testing strains cultures were added in each numbered wells containing 100 µL of broth medium (Sabouraud for yeast and Mueller-Hinton for bacteria) followed by 100 µL of resazurin solution and then 100µL of each serially diluted honey samples was added in well. After incubation in 37°C for 24 hours, the blue colored solution in microtiter plates indicates growth inhibition of bacteria. The negative control contained the sterile broth and resazurin solution, although the positive control is made up of all solutions with the exception of the test extract. Amphotericin B was used as positive control for fungal MICs determination.

1.3. Statistical analysis
Statistical analysis was performed using StatView 5.0 software. The experimental results were reported as mean ± SD (standard deviation) (n=3). Analysis of variance (ANOVA) and post-hoc Fisher PLSD ($p<0.05$) tests were used to compare the experimental groups. Pearson's correlation coefficient (R^2) was used to measure the association between two variables. Differences at $p< 0.05$ were considered significant.

RESULTS AND DISCUSSION

1.4. Total Phenolic and flavonoids content

The total phenolic content of the honey samples illustrated in **Table 1** showed a significant difference ($p < 0.05$) and varied widely from 239.47 mg GAE/kg for orange honey to 1138.53 mg GAE/kg for Thyme honey. Our results confirm previous results reported byAazza et al[14] for Moroccan honeys andLiberato et al[15] for Brazilian honeys who found that total phenolic content ranging between (102.1 - 1085 mg GAE/kg), (163.82- 923.70 mg GAE/kg) respectively. However, they are much lower than this reported byÖzcan and Olmez[16] for Turkish honeys who found phenolic content ranging between 170.06 - 885.43 mg GAE /100 g. The results reported by Moniruzzaman et al[17] for Malaysian honeys, which ranged between (144.51 - 580.03 mg GAE/kg) were found lower than the results reported in this study. Concerning total flavonoids content, the result depicted in the **Table 1** showed that it varied considerably between 11.72 mg for Orange honey and 179. 08 mg for Thyme honey in terms of Rutin equivalents/Kg of honey. The flavonoid content of honey samples analyzed in this study is highto those reported byAazza et al[14] for Moroccan honeys, but very close to results reported by the same author using Portuguese honey[18] . The difference within honeys samples may be owing to floral source, geographical area as well as the time of honey collection [17].

Table 1. Antioxidant activities, total phenolic content and total flavonoid of honey samples

	Total Phenolic (mg GA/Kg FW)	Total Flavonoid (mg RE / Kg FW)	FRAP µmol TE/Kg DW	ABTS µmol TE/Kg DW
Acacia	965.81±12.49	143.29±1.64	2516.36±32.8[a]	1983.48±31.74
Carob	652.82±5.31	66.17±1.02	1286.53±19.38	924.74±9.85
Eucalyptus	598.24±3.97	59.73±0.72	1207.41±15.42	1252.88±16.28
Harmal	838.73±4.21	92. 38± 0.44	1978.33±22.91	1716.87±14.88
Jujube	791.34±6.28	127.82±0.99	1844.73±23.72	1851.26±10.54
Lavender	546.71±5.52	44.26±0.31	1365.82±15.23	1031.17±13.66
Orange	239.47± 2.81	11.72± 0.63	744.45±11.62	634.37±8.28
Reseda	413.92±4.63	37.88±0.29	798.11±17.24	705.49±9.17
Rosemary	749.63±8.73	73.03±0.65	1634.18±29.17	1455.38±10.24[a]
Spurge	887.21±6.92	86.54±0.52	1766.04±46.84	1476.23±17.46[a]
Thyme	1138.53±11.03	179.08±1.37	2482.62±24.96[a]	2471.27±19.82

Values are mean of triplicate determinations (n = 3) ± standard deviation. The results are statistically significant at p < 0.001. GAE: Gallic acid equivalents. RE: Rutin equivalents. TE : Trolox equivalents; in the same column, the values with different letters are significantly different using post hoc PLSD Ficher (p < 0.001).

1.5. Antioxidant activities

The fact that the honey contains a wide range of phenolic compoundswith diverse antioxidant capacities requires the use of different antioxidant methods to better examine their antioxidant capacities.Hence, in this research, the ferric reducing ability (FRAP), free radical scavenging activity assays: ABTS, DPPHas well as β-carotene bleaching assay were used in this respect.

The ferric reducing antioxidant power (FRAP)is based on the reduction, at low pH, of a colorless ferric complex ferric-tripyridyltriazine $Fe(TPTZ)^{3+}$ to a blue-colored ferrous complex $Fe(TPTZ)^{2+}$ by the action of electron-donating antioxidants monitored by measuring the change of absorbance at 593 nm[19]. In contrast, the ABTS assay measured the reduction of the blue-green$ABTS^{+}$ radical by hydrogen-donating antioxidants[20].The level of discoloration monitored spectrophotometrically at 734 nm is proportional to scavenging activity of antioxidant. Both methods are easy to perform and usually used to assess antioxidant activity in foods. The results of antioxidant activities of honey samples based on FRAP assay and ABTS assay are given in **Table 1**. Acacia honey exhibited the highest level of antioxidant activity based on FRAP assay (2516.36 µmol TE/kg), while Thyme honey showed the highest level antioxidant activity based on FRAP assay (2371.27 µmol TE/kg). Orange honey exhibited the lowest antioxidant activity based on FRAP assay (644.45 µmol TE/kg) and ABTS assay (534.37 µmol TE/kg). The results established in this study are in agreement with those observed byGorjanović et al[21] for Serbian honeys who has found the antioxidant activities in the range of (200 to 4980 µmol TE/kg) and (1000 to 5820 µmol TE/kg) for FRAP assay and ABTS assay respectively.

The very strong correlation observed between FRAP/phenolic(R^2= 0.909), FRAP/Flavonoid (R^2= 0.876), ABTS/ phenolic (R^2= 0.880) and ABTS/ Flavonoid(R^2= 0.932) as showed**Table 3**, suggests that flavonoids and phenolics are the main contributor of these antioxidant activities and they facilitate the scavenging of free radicals by donating an electron or hydrogen atom.The strongest correlation (R^2= 0.908) was observed between these assays as showing**Table 3**. This relationship may be due to their similar redox potential for Fe^{2+}/ Fe^{3+} (0.70 V) and for $ABTS/ABTS^{+}$ (0.68V)[22]a bite higher potential redox of FRAP compared to ABTS may justify the higher antioxidant activities depicted using FRAP assay than ABTS assay, that means that any compound with lower Fe^{2+}/ Fe^{3+}redox potential can theoretically reduce Fe^{3+} to Fe^{2+} and contributes to the FRAP values resulting in falsely high FRAP values [19].

DPPH method is widely used assay to assess the scavenging ability in foods. It is based on the ability of antioxidant to provide hydrogen atoms or electrons to the stable radical DPPH formed in a liquid solution that possessed a purple color to become a stable diamagnetic molecule with yellow coloration, causing an absorbance decrease at 517nm [23].Whereasthe β-carotene bleaching assay was based on the ability of antioxidant to hinder the extent of b-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [24]. As seen from the data in **Table 2,** higher DPPH free radical scavenging activity (IC_{50}=5.52 mg/mL) was shown in thyme honey, which revealed the better antioxidant activity based on β-carotene bleaching assay (24.37 mg/mL). While orange honey exhibited the lowest antioxidant ability based on DPPH assay and (IC_{50}=14.61 mg/mL) and β-carotene bleaching assay (IC_{50}=79.08 mg/mL). The reported results of the DPPH assay were in close agreement with those reported by [25] who have found DPPH IC_{50} in the range of (3.17 to 8.79 mg/mL) for Brazilian honey. Strong correlation was observed between β-carotene bleaching assay/phenolic (R^2= 0.832), β-carotene bleaching assay/flavonoids(R^2= 0.626), DPPH assay/phenolic(R^2= 0.962), and DPPH assay/flavonoid(R^2= 0.847) as shown in**Table 3**. This strong correlation suggests that the free radical-scavenging ability can be attributed to the hydroxyl groups of phenolic and flavonoids, which can give the electron and neutralize the existing free radical in the reaction mixture. The involvements of free radicals in the pathogenesis of a large number of diseases are well-documented [26].Hence, an effective scavenger of these free radicals may serve to prevent diseases.

Table 2. DPPH scavenging power and β-carotene bleaching inhibition activity of honey samples

	β-carotene bleaching assay IC_{50} (mg/mL)	DPPH assay IC_{50} (mg/mL)
Acacia	34.96±0.15	6.68±0.10
Carob	51.23±0.44	11.96±0.18[a]
Eucalyptus	58.84±0.61	10.73±0.15
Harmal	45.16±0.46	8.52±0.11
Jujube	61.92±0.58	9.29±0.14
Lavender	49.42±0.87	11.84±0.17[a]
Orange	79.08±0.62	15.61±0.26
Reseda	70.59±0.57	12.53±0.16
Rosemary	54.61±0.69	9.93±0.10
Spurge	39.59±0.36	7.91±0.08
Thyme	24.37±0.28	5.52±0.09

Values are mean of triplicate determinations (n = 3) ± standard deviation. The results are statistical ly significant at (p < 0.001). In the same column, the values with different letters are significantly different using post hoc PLSD Fisher.

Table 3.Correlation phenolic and flavonoid content with antioxidant activities

	Phenolic	Flavonoid	ABTS	FRAP	DPPH	β-Carotene
Phenolic	1					
Favonoid	0.872	1				
ABTS	0.880	0.932	1			
FRAP	0.909	0.876	0.908	1		
DPPH	0.962	0.847	0.878	0.891	1	
β-Carotene	0.832	0.626	0.628	0.764	0.793	1

1.6. Antibacterial activity

1.6.1.Disc Diffusion assay

Six standard strains bacteria, including three Gram positive: *S. aureus B. subtilis, S. pyogenes* and three Gram negative:*E. coli, P. aeruginosa, S. abony* was selected for the antibacterial screening of eleven honey samples. The results of thedisc diffusion analysis shown in **Table 4** displayed that all tested honeys possessed an antibacterial activity against the test strains. Overall, the honey samples showed maximum significant antibacterial activity against *S. aureus*, however the less significant antibacterial activity was shown against *P. aeruginosa*.

Thyme honey exhibited the highest antibacterial activity against *B. subtilis* (ZID=23.9mm), *S. pyogenes* (ZID=23.5mm) and *P. aeruginosa* (ZID=17.3mm) and spurge honey was found to be the highest inhibitor of *E.coli* growth (ZID=22.4mm).The highest antibacterial activity against *S. aureus* (ZID=24.6mm),*S.abony* (ZID=16.9mm) were found usingAcacia honey. Among analyzed honeys, orange honey possessed the lowest antibacterial activity against all bacterial strains with the lowest zone inhibition diameter. All bacterial strains except *S. pyogenes* showed a significant sensitivity for gentamicin (10µg/disc) in the range of (17.66 to 19.66 mm).Our results are in agreement with those reported byRayes [27] but lower than those reported by Wilkinson and Cavanagh[28] and Hamouda and Marzouk[29].

This data about analysis show that the gram positive bacterial strains are more sensitive ofhoney samples than the gram negative bacterial strains.Previous researches also support this finding [30, 31].This might be due to ability of outer membrane in gram-negative bacterial cell to exclude certain antibiotic compounds from penetrating the cell.

1.6.2.Minimum Inhibitory Concentration (MIC)

After the antibacterial screening using the disc diffusion assay, all honey samples revealed a positive result against the test pathogens hence further studies for the determination of MIC are required. **Table 5** summarized the data of MICsof each honey sample against test bacterial strain. Thyme honey gave the highest antibacterial activity against *B. subtilis* (6.00 mg/mL), *S. pyogenes* (7.33 mg/mL), *E. coli* (7.66 mg/mL) and*P. aeruginosa* (9.50 mg/mL). The maximum antibacterial activity against*S. aureus* (5.16 mg/mL) *S.abony* (7.83 mg/mL) were found using Acacia honey. Amongst the test bacterial strains,*S. aureus* was the most sensitive and *P. aeruginosa* was less sensitive. The MICs of gentamicin against these bacterial strains still higher than the MICsof study honey samples. The results of MICs reported in this study are higher than those reported byChauhan et al[32],who found the MICs in the range of (0.625–5.000 mg/mL), for *S. aureus*, *B. subtilis*, *B. cereus*, and gram-negative bacteria (*E. coli*, *P. aeruginosa* and *S. typhi*). But lower than the results of MICs reported byBoateng and Diunase[33] who was found 10%v/v as MIC for *E. coli*, *P. aeruginosa* and *S.aureu.*The difference observed in the antimicrobial activity among studied honeys and compared to other honey samples studied in several investigations might be due to dissimilarities of honey origin and processing, honeybees, floral source and season of harvesting. The source of bacterial strains, its inoculum size and its growth speed as well as the test method may also be critical in this respect [34].The antibacterial activity of honey has been attributed to its high osmotic potential, hydrogen peroxide content, high natural acidity, its content of phenolic, flavonoid, ascorbic acid, amylase, fatty acids, trepens, sulfathiazole, benzyl alcohol and benzoic acids, streptomycin and its content of tetracycline derivatives [32]. This antibacterial activity may be attributed also to its methylglyoxal content known for its antibacterial property, which was found in high concentration in Manuka honey [35].In our study, this activity appeared to be influenced by phenolic and flavonoid composition as shown the highest positive correlation coefficient between phenolic and flavonoid content in one hand and antibacterial activity in the other hand (**Table 7**).This is in accordance with previous studies that demonstrate a significant correlation between phenolic and flavonoid composition and antimicrobial activity[36].

Table 5. Determination of Minimum inhibitory concentration of bacterial growth

	Minimum inhibitory concentration MIC mg/mL					
	Gram positive bacteria			Gram negative bacteria		
	S. aureus	*B. subtilis*	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*S. abony*
Acacia	5.16±0.22^{d}	6.17±0.22^{e}	8.00±0.33^{de}	8.66±0.22^{e}	10.33±0.22^{d}	7.83±0.22^{h}
Carob	8.83±0.22^{a}	7.83±0.22c	9.33±0.22	11.83±0.22^{a}	11.83±0.22^{bc}	9.33±0.22^{de}
Eucalyptus	8.5±0.33^{a}	8.33±0.22^{b}	9.83±0.22	10.66±0.22^{b}	11.50±0.33^{c}	10.50±0.33^{b}
Harmal	6.50±0.33^{b}	7.5±0.00^{d}	9.00±0.00	9.50±0.33^{cd}	10.5±0.33^{d}	9.16±0.22^{e}
Jujube	6.83±0.22^{b}	7.33±0.22^{d}	8.66±0.22c	9.16±0.22^{de}	10.66±0.22^{d}	8.83±0.33^{ef}
Lavender	9.33±0.22	8.5±0.33^{ab}	10.33±0.22^{b}	10.83±0.22^{b}	12.50±0.33^{a}	10.33±0.22^{bc}
Orange	10.83±0.22	9.83±0.22	11.00±0.33^{a}	12.5±0.33	13.83±0.22	12.00±0.33^{a}
Reseda	9.83±0.22	8.5±0.22^{ab}	10.5±0.33^{ab}	11.50±0.33^{a}	12.16±0.22^{ab}	11.66±0.22^{a}
Rosemary	7.5±0.33	7.5±0.33^{cd}	8.50±0.33^{cd}	9.83±0.22^{c}	11.00±0.00	9.83±0.33^{cd}
Spurge	5.83±0.22^{c}	6.67± 0.22	8.00±0.00^{e}	8.16±0.22	9.83±0.22e	8.5±0.33^{fg}
Thyme	5.50±0.33^{cd}	6.00±0.00^{e}	7.33±0.22	7.66±0.22	9.50±0.33e	8.00±0.33^{gh}

Values are mean of triplicate determinations (n = 3) ± standard deviation. The results are statistically significant at (p < 0.001). In the same column, the values with different letters are significantly different using post hoc PLSD Fisher.

Table 4. Effect of honey samples on Growth Inhibition of Bacteria and Fungi

	Disc inhibition zone (mm) (15%)						Disc inhibition zone (mm) (35%)		
	Gram positive bacteria			Gram negative bacteria			Yeasts		
	S. aureus	***B. subtilis***	***S. pyogenes***	***E. coli***	***P. aeruginosa***	***S. abony***	***R. mucilaginosa***	***C. neoformans***	***C. albicans***
Acacia	24.64±1.09^{a}	21.61±1.33^{a}	21.69±0.58	20.82±0.65^{b}	15.84±0.52^{bc}	16.91±0.83^{a}	6.52±0.38^{bc}	8.62±0.49^{d}	5.16±0.25^{cd}
Carob	14.72±0.64^{d}	16.55±0.71^{de}	16.71±0.89^{bc}	12.27±0.78^{g}	12.45±0.74^{e}	13.66±0.56^{de}	3.16±0.42	4.58±0.46^{a}	ND
Eucalyptus	15.49±0.72^{d}	15.62±0.46^{ef}	15.53±0.46^{cd}	15.69±0.72^{e}	13.68±0.46^{d}	11.83±0.60^{f}	ND	4.86±0.34^{ab}	ND
Harmal	20.75±0.81^{b}	17.94±0.39^{c}	17.62±0.52^{b}	18.83±1.21^{cd}	15.09±0.49^{c}	14.38±0.46^{cd}	5.83±0.41^{ab}	7.23±0.29^{c}	4.86±0.31^{bc}
Jujube	19.16±0.48^{c}	18.87±1.21^{bc}	18.93±0.72^{a}	19.35±0.42^{c}	15.29±0.95^{bc}	14.77±0.82^{bcd}	7.27 ±0.53^{c}	7.54±0.48^{c}	4.33±0.42^{ab}
Lavender	14.42±1.28^{de}	14.11±0.53^{g}	14.80±0.85^{de}	14.94±1.01^{ef}	11.76±0.54^{e}	12.52±0.78^{ef}	ND	4.42±0.51^{a}	ND
Orange	11.37±0.52	12.83±0.67	12.21±0.31	10.48±0.52	6.71±0.29	9.19±0.45	ND	ND	ND
Reseda	13.12±0.59^{e}	14.74±0.66^{fg}	13.56±0.62^{e}	13.76±0.75^{fg}	12.13±0.46^{e}	10.62±0.29	ND	ND	ND
Rosemary	18.44±0.76^{c}	17.26±0.54^{cd}	18.37±0.96^{ab}	17.08±0.56^{d}	14.57±0.82^{cd}	13.31±0.84^{de}	5.34±0.37^{a}	6.36±0.28	3.59±0.57^{a}
Spurge	22.53±1.19^{ab}	20.73±1.02^{ab}	20.44±0.54	22.47±0.63^{a}	16.48±0.67^{ab}	15.26±0.52^{bc}	4.96±0.57^{a}	5.48±0.39^{b}	3.83±0.46^{a}
Thyme	23.48±0.82^{a}	23.91±0.67	23.52±0.71	21.66±1.24^{ab}	17.30±0.68^{a}	16.52±0.97^{ab}	7.42±0.61^{c}	8.69±0.46^{d}	5.57±0.19^{d}
Gentamicin	18.00±0.00	19.66±0.33	Nd	19.00±0.00	17.66±0.33	18.66±0.22	-	-	-

Values are mean of triplicate determinations (n = 3) ± standard deviation. The results are statistically significant at (p < 0.001). In the same column, the values with different letters are significantly different using post hoc PLSD Fisher. ND: Activity not present.

1.7. Antifungal activity

1.7.1.Disc Diffusion assay

In this study, we have tested eleven honey samples for their antifungal activity against three yeast strains *C. albicans, C. neoformans and R. mucilaginosa* commonly isolated from clinical specimens. The results of zone inhibition diameter (ZID) determination are given in **Table 4**. The highest antifungal activity was found for thymehoney against*C. neoformans* with a highest ZID (8.70mm) this fungiwas found the most sensitive to all honey

samples and the lowest antifungal activity was found inCarob honey against *R. mucilaginous* with the lowest ZID (3.16 mm). *C. albicans* was the most resistant of testing yeast strains. Among analyzed honeys, only six samples showed an antifungal activity. Orange and Reseda honeys did not show anyactivity against tested fungi. The results of our study are very close to the result of Moussa et al[37], buta bit higher than those reported by Anyanwu[38].The fact that honey samples did not show the same level of activity against tested fungal strains as shown the result suggest that physical-chemical properties, botanical origin and entomological origin play an important role in influencing the antimicrobial activity. Varying sensitivities of *Candida albicans* to different honey samples was observed also by Anyanwu[38], Alzahrani et al[39]. The emergence of resistant strains may be the reason of the low susceptibility of some of the test organisms in the honey samples.

1.7.2.Minimum Inhibitory Concentration (MIC):

The honey samples, which showed a positive result against all testfungi were used to determine the MIC. **Table 6** shows the result of MIC of honey samples against three tested fungi. Thehighest antifungal activity was observed for thyme honey against *C. neoformans*(MIC=15.16%) and the lowest was revealed in spurge honey against *C. albicans*(MIC=29.50%). Our results are in agreement with those reported byAnyanwu[38] who has found using four honey samples against six tested fungal strainsMICs a range of (20-25%), (20%), (20%), (12.5-20%), (20-25%) and (20-40%) for *A. niger*, *A.flavus*, *P.chrysogenum*, *M.gypseum*, *C.albicans*, and Saccharomyces spp respectively[40] has found (MIC= 19.33%) for *C. neoformans*, and (MIC= 23.33%) for *C. albicans*, which are very close to our results.The analysis of correlation (**Table 7**) between MIC versus flavonoids and MIC versus phenols shows a medium to high correlation between MIC and phenolic content, and very strong correlation was observed between MIC and flavonoids suggesting that these compounds may be the main contributor in honey antifungal activity. Candiracci et al[41] have demonstrated that flavonoids present in honey have the capacity to inhibit the dimorphic conversion of *C. albicans*.

Table 6. Determination of Minimum inhibitory concentration of fungal growth

	Minimum inhibitory concentration MIC %		
	R. mucilaginosa	***C. neoformans***	***C. albicans***
Acacia	19.00 ± 0.33	16.66±0.33	21.33±0.22
Harmal	19.83 ± 0.22	17.83±0.22	26.50±0.33
Jujube	18.33 ± 0.22	18.50±0.33	24.83±0.22
Rosemary	20.66 ± 0.22	19.83± 0.22	27.83±0.22
Spurge	24.50 ± 0.33	21.66± 0.22	29.50±0.33
Thyme	16.83 ± 0.22	15.16±0.22	23.66±0.22
Amphotericin B	0.000005	0.000007	0.00001

Values are mean of triplicate determinations (n = 3) ± standard deviation. The Results are statistically significant at (p < 0.001). In the same column, the values with different letters are significantly different using post hoc PLSD Fisher.

Table 7. Correlation phenolic and flavonoid content with antibacterial and antifungal activities

	S. aureus	*B. subtilis*	*S. pyogenes*	*E. coli*	*P. aeruginosa*	*S. abony*	*R.mucilaginosa*	*C.neoformans*	*C.albicans*
Phenolic	0.928	0.948	0.937	0.868	0.918	0.912	0.777	0.809	0.784
flavonoid	0.800	0.838	0.808	0.760	0.751	0.804	0.947	0.845	0.517

CONCLUSION

The results of this work have shown that honey samples revealed considerable antibacterial and antifungal activities. Moreover, the studied honeys exhibited a high reducing activity, high inhibition of lipid peroxidation as well as a high scavenging activity. These activities were found in high correlation with their phenolic and flavonoid contents. The current investigation proved the traditional use of honeys for numerous human ailments especially for infectious diseases. Hence, it could be recommended to use the honey as an alternative of antimicrobial drugs.

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