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Research Article

# Biological Activities of Extracts of Different Spices and Plants

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***Abstract***

*Aqueous, alcoholic and hexanic extracts of different spices and plants materials were investigated for their antioxidant and antibacterial activities. The total flavonoid contents of extracts varied from 1.65 µg quercetin equivalent/mg (water extract of red pepper) to 340.13 µg quercetin equivalent/mg (alcoholic extract of miswak). The aqueous extract of black tea and the ethanolic extracts of both cinnamon and clove displayed the highest total phenolic contents with 196.57, 194.06 and 198.16 µg gallic acid equivalent/mg, respectively. Antioxydant activities of extracts were assessed using DPPH-free radical scavenging method. Ethanolic and aqueous extracts of cinnamon, ethanolic extract of clove and aqueous extract of coffee displayed the strongest antioxidant activity with $IC_{50}$ values of 1.45, 1.96, 2.16, 3.02 and 5.4 µg/ml, respectively. Antibacterial activities of extracts against several bacterial genera, known to be pathogenic to humans, were investigated using the agar well diffusion method. The hexanic extracts were effective against most of the microorganisms tested. Particularly, S. xylosus was found to be highly sensitive to most of the hexanic extracts and especially to those of miswak, clove and turmeric with minimal inhibitory concentrations values of 15.62 and 31.25 and 62.5 µg/mL, respectively.*

***Keywords:*** *spices, plants, antibacterial, antioxidant, total phenolics, total flavonoids*

## 1. Introduction

The use of spices and herbs for their flavouring, preservative and health promoting properties has been known since ancient times. Early records indicate that they were used as medicinal in ancient Egypt and Assyria and as food preservatives in ancient Rome and Greece (Kaefer and Milner, 2008).

Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry, because of their strong antioxidant and antimicrobial properties, which exceed many currently used antibiotics and synthetic antioxidants (Mathew and Abraham, 2006). These properties are due to the presence of several substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens and minerals (Calucci *et al*, 2003).

In fact, development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the antibacterial compounds in plants (Duman-Aydin, 2008; Pokhrel *et al*, 2012). In addition, currently available synthetic antioxidants like butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinone (TBHQ) and gallic acid esters have been suspected to cause or prompt negative health effects such as carcinogenicity (Zheng and Wang, 2001). Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990; Branen, 1975).

Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in foods (Pryor, 1991; Kinsella *et al*, 1993; Lai *et al*, 2001). In the past decades, oxidation mechanisms and free radicals role in living systems have gained increased attention. Reactive oxygen species (ROS) are produced as a by-product of cellular metabolic pathways and function as a critical second messenger in a variety of intracellular signaling pathways. The reaction of this species with lipid molecules originates peroxyl radicals and their interaction with nucleic acids and proteins conduces to alterations and, therefore, to functional modifications (Chaillou and Nazareno, 2006). ROS are continuously produced during normal physiologic events and can easily initiate the peroxidation of membrane lipids, leading to the accumulation of lipid peroxides. ROS is capable of damaging crucial biomolecules such as nucleic acids, lipids, proteins and carbohydrates. Also, ROS and reactive nitrogen species (RNS) may cause DNA damage that may lead to mutation and to disease occurrence (Gulcin *et al*, 2005, Gulcin *et al*, 2006). ROS are exacerbating factors in

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**Table 1** List of spices and plants used in this study with their correspondent extraction yield (% dry weight base). AE, Aqueous Extract; EE, Ethanolic Extract and HE, Hexanic Extract

| NO. | Name | Scientific name | Extraction part | Extraction yield (%) | | |
|---|---|---|---|---|---|---|
| | | | | AE | EE | HE |
| 1 | Anise | *Pimpinella anisum* | Seeds | 10 | 14.25 | 10.55 |
| 2 | Bay laurel | *Laurus nobilis* | Leaves | 12.5 | 13.77 | 8.32 |
| 3 | Black pepper | *Piper nigrum* | Corns | 10 | 12.75 | 5.5 |
| 4 | Red pepper | *Capsicum annuum* | Fruit | 22.5 | 20.02 | 15.55 |
| 5 | Chili pepper | *Capsicum frutescens* | Fruit | 17.5 | 24.47 | 17.35 |
| 6 | Black Tea | *Camellia sinensis* | Leaves | 5.32 | 5.32 | 3.65 |
| 7 | Green Tea | *Camellia sinensis* | Leaves | 7.5 | 3.42 | 4.77 |
| 8 | Caraway | *Carum carvi* | Seeds | 10 | 15.4 | 18.55 |
| 9 | Cinnamon | *Cinnamomum verum* | Bark | 4.77 | 16.9 | 4.07 |
| 10 | Clove | *Syzygium aromaticum* | Flowers | 27.5 | 3.37 | 2.6 |
| 11 | Coffee | *Coffea arabica* | Beans | 20 | 14 | 12.45 |
| 12 | Common Vervain | *Verbena officinalis* | Leaves | 7.77 | 7.6 | 3.62 |
| 13 | Corchorus | *Corchorus olitorius L.* | Leaves | 10 | 6.42 | 2.5 |
| 14 | Coriander | *Coriandrum sativum* | Seeds | 20 | 11.87 | 10.6 |
| 15 | Cubeb | *Piper Cubeba* | Seeds | 10 | 8.02 | 6.45 |
| 16 | Cumin | *Cuminum cyminum* | Seeds | 20 | 24.42 | 23.87 |
| 17 | Fennel | *Foeniculum vulgare* | Seeds | 12.5 | 10.92 | 11.67 |
| 18 | Ginger root | *Zingiber officinale* | Rhizome | 15 | 7.5 | 20.5 |
| 19 | Mint | *Mentha aquatica* | Leaves | 15 | 10.82 | 3.62 |
| 20 | Miswak | *Salvadora persica* | Bark | 5 | 7.65 | 3.1 |
| 21 | Nigella | *Nigella sativa* | Seeds | 10 | 41.9 | 24.55 |
| 22 | Orange peel | *Citrus sinensis* | Peel | 27.5 | 19.2 | 1.25 |
| 23 | Safflower | *Carthamus tinctorius* | Flowers | 10 | 15 | 24.02 |
| 24 | Turmeric | *Curcuma longa* | Rhizome | 5 | 7.22 | 3.92 |

cellular injury and aging process (Gulcin *et al*, 2002), prostate and colon cancers, coronary heart disease, atherosclerosis, (Madhavi *et al*, 1996), Alzheimer's disease, diabetes mellitus, hypertension and AIDS (Wong *et al*, 2006; Mosquera, 2007).

A large number of reports concerned with the antioxidative and antibacterial activity of spices and plants have been published. Comparison of the results of different experiments is often complicated by the fact that the antioxidant and antibacterial activity of a specific spice varies according to the country in which it was grown, to the extraction techniques and to the substrate used in the evaluation. Within this context, various solvent extracts (i) from several spices commonly used in Tunisian cooking (ii) from coffee, green and black teas which are aromatic beverages habitually drunk in many countries and (iii) from miswak, a teeth cleaning twig reputed for its medicinal benefits, were studied using the same procedure and technique. The extraction yield and the total phenols and flavonoids contents were evaluated and antioxidant and the antibacterial potentials were investigated.

## 2. Material and methods

### *2.1. Chemical reagents and standards*

To perform experiments, several materials was provided. In fact, 1,1 diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Folin-Ciocalteu reagents and ampicillin were purchased from Sigma chemicals (Steinheim, Germany).

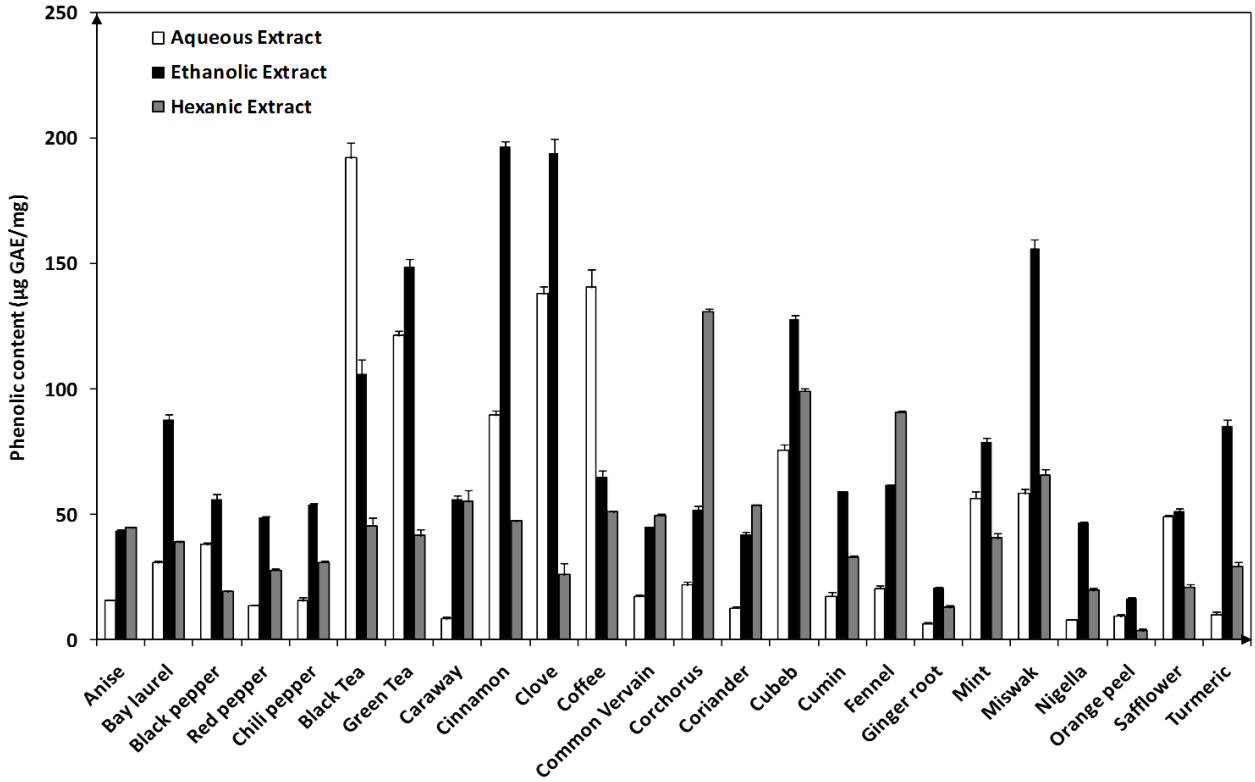


**Fig. 1** Phenolic contents obtained in various solvent extracts. The values are expressed as micrograms gallic acid equivalent/mg of spice powder (µg GAE/mg).

Butylated hydroxytoluene (BHT) was purchased from Prolabo (Paris, France). Gallic acid and quercetin were obtained from Fluka (Buchs, Switzerland). All other chemicals and solvents were of analytical grade.

*2.2. Plant material*

All samples (spices and plants) (Table 1) are purchased from a local market (Sfax-Tunisia). They were finely powdered using a grinder (Braun-Germany) and stored at 4C° until use.

*2.3. Preparation of crude extracts*

Each spice powder (5 g) was extracted by maceration technique using 50 ml of solvents with different polarities (Hexane, ethanol and water). After 24 hours, the suspensions were filtered through a Buchner funnel. The hexanic and the alcoholic extracts were concentrated using a rotary evaporator (Buchi Rotavapor R-200) at 50C°. The aqueous extract was lyophilized and the extraction yield was calculated based on the dry weight of the spice. The resulting powder was packed in a glass bottle and stored at 4C°, until needed.

*2.4. Total phenolic contents*

The total amounts of phenolic compounds were determined by the Folin-Ciocalteu reagent as previously described (Slinkard and Singleton, 1977). A volume of 0.5 ml of each extract was mixed with 0.5 ml of the Folin-Ciocalteu reagent. After 5 min, 0.5 ml of 20% sodium carbonate ($Na_2CO_3$) solution was added and the solution was brought up to 5 ml by adding distilled water. After a 90 min-incubation at room temperature in darkness, the absorbance was measured at 760 nm. Gallic acid was used as standard reference. The concentrations of total phenolic compounds in the different spices extracts were determined as micrograms of gallic acid equivalent/mg of spice powder (µg GAE/mg). The equation obtained from the standard gallic acid graph is as follows:

Absorbance = 0,006 x µg gallic acid – 0.021 ($R^2$ = 0.99).

*2.5. Total flavonoid content*

The total flavonoid content was determined as previously described (Zhishen *et al*, 1999). An aliquot of each sample (250 µl) was mixed with 1 ml of distilled water and subsequently with 150 µl of 150 mg/ml sodium nitrite solution. After a 6 min incubation, 75 µl of 100 mg/ml aluminium chloride solution was added, and then the mixture was left 5 min before the addition of 1ml of 40mg/ml sodium hydroxide (NaOH) solution. Distilled water was immediately added to the mixture until the total volume reached 2.5 ml. The absorbance was measured at 510 nm. Quercetin was used as a reference compound. The total flavonoid content was calculated and expressed as micrograms quercetin equivalent/mg of spice powder (µg QE/mg). The calibration recorded for this standard was expressed as follows: Absorbance = 0.006 µg quercetin + 0.006 ($R^2$ = 0.99).

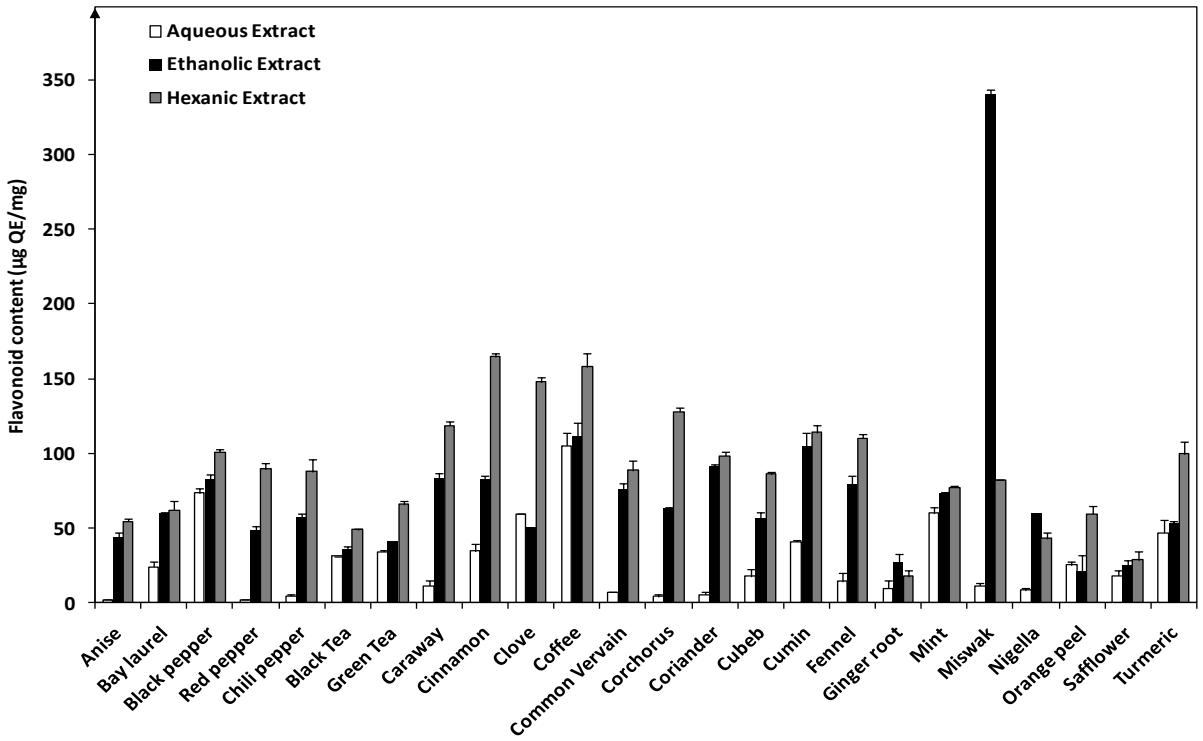


**Fig. 2** Flavonoid contents obtained in various solvent extracts. The values are expressed as micrograms quercetin equivalent/mg of spice powder (µg QE/mg).

*2.6. DPPH radical-scavenging assay*

1,1 Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay was determined according to Brand-Williams et al. (1995). A stock solution (10 mg/ml each) of solvent extracts was prepared in absolute ethanol. All samples were used at concentrations in the range of 1-1000 µg/ml. A volume of 500 µl of each sample was mixed with 500 µl ethanol and 125 µl of freshly prepared solution of 0.02% DPPH in 100% ethanol. The mixture was shaken vigorously and incubated at room temperature in darkness during 60 min. The absorbance of the remaining DPPH radicals was read at 519 nm using a Shimadzu UV mini-1240 UV-VIS spectrophotometer (Paris, France). The scavenging of DPPH radical was calculated according to the following equation:

$$\text{DPPH Radical-scavenging activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100$$

where $A_{control}$ and $A_{sample}$ are the absorbance of the control and the sample, respectively. Then inhibition percentages were plotted against respective concentrations used and $IC_{50}$ was calculated from the graph. The BHT was used as positive control. The values were presented as the means of triplicate analysis.

*2.7. Antibacterial activity*

In order to determine the antibacterial activity of the different solvent extracts, various Gram-positive and Gram-negative bacteria were used: *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 27853), *Salmonella enteric* (ATCC 43972), *Enterobacter cloacae* (ATCC 13047), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 14990) and *Brevibacterium flavum* (ATCC 14067). *Staphylococcus xylosus* was isolated in LBGEL from waste water (Mosbah *et al*, 2005).

*2.7.1. Agar well diffusion method*

The agar well diffusion method was used to evaluate the antibacterial activities of the solvent extracts according to the method described by Berghe and Vlietinck (1991). The dried extracts were dissolved in 100% ethanol to a final concentration of 20 mg/ml. The bacterial strains were cultured in a nutriment broth for 24 h. Then, 200 µl of each suspension bacteria ($10^6$ colony-forming unit estimated by absorbance at 600 nm) was spread on Luria Broth agar. Bores were made by using a sterile borer and were loaded with 20 µl of each sample extract. Ethanol was used as negative control and ampicillin (10 µg/well) as positive reference standard. All the plates were incubated at 37 C° for 24 h. Antibacterial activity was evaluated by measuring in millimeters the zone of inhibition. All experiments were done in triplicates.

*2.7.2. Determination of the minimum inhibitory concentration (MIC)*

The Minimal Inhibitory Concentrations (MIC) of each extract against the tested microorganisms was determined by the broth microdilution method (Mims *et al*, 1993). The final concentration of bacteria in each macrobroth dilution tube was approximately $6.8\times10^6$ CFU/mL of LB. The MIC was defined as the lowest concentration of the extract

**Table 2** DPPH free-radical scavenging activity (CI50) of various solvent extracts. AE, Aqueous Extract; EE, Ethanolic Extract and HE, Hexanic Extract

| NO. | Name | $IC_{50}$ (µg extract/mL) | | |
|---|---|---|---|---|
| | | AE | EE | HE |
| 1 | Anise | 37.15± 2.05 | 51.97± 2.15 | 308.19± 12.18 |
| 2 | Bay laurel | 73.60± 4.15 | 7.05± 2.15 | 233.28± 10.48 |
| 3 | Black pepper | 92.28± 3.12 | 98.95± 6.33 | 134.40± 8.05 |
| 4 | Red pepper | 150.38± 9.63 | 324.14± 9.47 | 759.56± 16.68 |
| 5 | Chili pepper | 122.52± 8.15 | 128.59± 6.25 | 782.44± 17.00 |
| 6 | Black Tea | 7.71± 1.32 | 4.34± 1.24 | 333.64± 9.58 |
| 7 | Green Tea | 24.88± 2.15 | 17.01± 1.34 | 68.48± 6.24 |
| 8 | Caraway | 181.29± 7.75 | 50.59± 6.84 | 188.97± 7.15 |
| 9 | Cinnamon | 1.96± 0.50 | 1.45± 0.25 | 386.40± 7.85 |
| 10 | Clove | 111.24± 3.85 | 2.16± 0.89 | 478.15± 9.75 |
| 11 | Coffee | 3.02± 0.23 | 133.94± 5.17 | 1224.89± 20.08 |
| 12 | Common Vervain | 126.73± 4.54 | 10.84± 2.11 | 663.27± 11.54 |
| 13 | Corchorus | 512.18± 12.10 | 111.24± 8.71 | 142.53± 8.03 |
| 14 | Coriander | 103.95± 8.17 | 84.61± 6.32 | 788.02± 16.47 |
| 15 | Cubeb | 5.55± 1.42 | 7.58± 1.42 | 13.35± 2.15 |
| 16 | Cumin | 77.87± 2.15 | 245.86± 5.71 | 717.27± 12.75 |
| 17 | Fennel | 20.34± 2.62 | 188.61± 4.41 | 419.28± 12.65 |
| 18 | Ginger root | 140.41± 10.25 | 108.06± 7.08 | 370.28± 17.17 |
| 19 | Mint | 18.51± 3.45 | 29.46± 2.15 | 16.16± 2.85 |
| 20 | Miswak | 57.23± 2.06 | 7.26± 2.15 | 6.87± 2.55 |
| 21 | Nigella | 127.83± 8.20 | 761.72± 13.25 | 146.44± 11.02 |
| 22 | Orange peel | 129.52± 7.15 | 273.18± 14.85 | 216.70± 10.25 |
| 23 | Safflower | 26.16± 2.70 | 33.78± 3.07 | 68.59± 3.52 |
| 24 | Turmeric | 332.86± 15.15 | 13.23± 2.45 | 421.44± 17.25 |

compound that resulted in no visible growth after 24 h of incubation at 37 C°. The MIC's were determined for all strains which showed significant zones of inhibition (more than 15 mm). Tests were performed in duplicates.

*2.8. Statistical analysis and correlation study*

Experimental results were given as mean value ± SD of three separate experiments. Statistical analysis was conducted using Microsoft Excel software. Differences at $P < 0.05$, using student's *t*-test, were considered to be significant.

## 3. Results and Discussion

*3.1. Extraction yields and total phenolic and flavonoid contents*

Spices are aromatic and pungent food ingredients that can be added to food in several forms: as whole spices, as ground spices, or as extracts. Therefore, their direct use as antioxidants is limited. The extraction procedure is determined by the types of antioxidant compounds to be extracted. Selection of a suitable extraction procedure can increase the antioxidant concentration relative to the plant material. For polyphenols and other antioxidants in plant materials, three principal extraction techniques may be used: extraction using solvents, solid-phase extraction and supercritical extraction (Suhaj, 2006). In this study, solvent extraction was adopted. Ethanol and hexane would probably be better than acetone, chloroform and methanol as eventual solvent residues would be less toxic (Karadeniz *et al*, 2005). Thus, hexane, ethanol and water which have various polarities, were used in this study.

The extraction yields using water or ethanol resulted in the highest amount of total extractable compounds whereas the lowest yields were obtained with hexane (Table 1). These variations can be attributed to the differences in polarities of the compounds present in the spices and plants used. The highest yield (41.9%) was obtained for the ethanolic extract of nigella followed by aqueous extract of clove (27.5%) and the lowest yield (1.25%) corresponded to the hexanic extract of orange peel.

It has been reported that phenolic and flavonoid compounds show antioxidant and antimicrobial activities and have good effects on human nutrition and health (Cook and Samman, 1996; Duman-Aydýn, 2008). The total phenolic and flavonoid contents of extracts were expressed as equivalent of gallic acid (µg GAE) and quercetin equivalent (µg QE) per mg of spice powder, respectively. The phenolic and flavonoid compounds levels were significantly different for the various extracts ($P$ <0.05). In general, the highest levels of the total phenolic contents were found in alcoholic extracts followed by the hexanic then the aqueous ones (Figure 1).

**Table 3** Inhibition zone of various solvent extracts (400 µg/well). AE, Aqueous Extract; EE, Ethanolic Extract and HE, Hexanic Extract

| Name | Anise | | | Bay Laurel | | | Black pepper | | | Red pepper | | | Chili pepper | | | Black Tea | | | Green Tea | | | Caraway | | | Cinnamon | | | Clove | | | Coffee | | | Common Vervain | | | Corchorus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial strains | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE |
| ***Gram (-)*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. coli* | - | + | + | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | + | + | + | - | - | + | + | + | + | - | - | - | - | - | - | - | - |
| *E. cloacae* | - | + | + | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | + | + | + | - | - | + | - | + | ++ | - | - | + | - | - | - | - | - |
| *K. pneumoniae* | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - | + | - | + | + | - | + | + | - | - | - | - | - |
| *S. typhimurium* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | + | + | - | - | ++ | - | - | - | - | - | - | - | - | - | - | - |
| ***Gram (+)*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. flavum* | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | + | - | - | + | - | + | + | - | - | - | - | - | - | - | - |
| *S. xylosus* | - | - | - | - | - | - | - | + | - | - | + | ++ | + | - | + | - | - | - | - | - | - | + | + | ++ | - | + | ++ | - | - | - | - | - | - | - | - | + | - | - |
| *S. aureus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | + | - | - | - |
| *S. epidermidis* | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | + | - | - | - | - | + | - | - | - | - | - | - | - | - | ++ | - | - |

| Name | Coriander | | | Cubeb | | | Cumin | | | Fennel | | | Ginger root | | | Mint | | | Miswak | | | Nigella | | | Orange peel | | | Safflower | | | Turmeric | | | Ampicillin | Ethanol |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial strains | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | AE | EE | HE | 10 µg /well | 20 µl |
| ***Gram (-)*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *E. coli* | - | - | + | - | - | + | - | - | + | - | - | + | - | - | + | - | - | - | - | + | ++ | - | - | - | - | - | - | - | - | - | - | - | - | +++ | - |
| *E. cloacae* | - | - | - | - | - | + | - | - | - | - | - | - | - | + | + | - | - | - | - | + | ++ | - | - | - | - | - | - | - | - | ++ | - | - | - | ++ | - |
| *K. pneumoniae* | - | - | - | - | - | - | - | - | - | - | - | - | ++ | + | - | - | - | - | - | + | ++ | - | - | - | + | ++ | - | - | + | - | - | - | - | +++ | - |
| *S. typhimurium* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | + | - | - | - | - | - | +++ | - |
| ***Gram (+)*** | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| *B. flavum* | - | + | + | - | - | + | - | + | - | - | + | + | - | - | + | - | + | ++ | - | + | ++ | - | - | - | + | + | + | - | - | - | - | - | - | ++ | - |
| *S. xylosus* | - | + | + | - | ++ | + | - | - | - | - | - | + | - | - | ++ | - | + | ++ | - | + | ++ | - | - | - | - | + | + | - | + | ++ | - | - | - | ++ | - |
| *S. aureus* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | + | ++ | - | - | - | - | - | - | - | - | - | - | - | - | ++ | - |
| *S. epidermidis* | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | + | - | - | ++ | - | + | + | - | - | - | - | - | - | - | - | - | +++ | - |

The bactericidal level was estimated by measuring the size of inhibition zone of the indicator strain. Insensitivity (-), low sensitivity (+: Diameter of inhibition < 15 mm), high sensitivity (++: Diameter of inhibition between 15 and 20 mm) and very high sensitivity (+++: Diameter of inhibition > 20 mm).

**Table 4** Minimal inhibitory concentration: MIC (µg/ml soluble extract) of various solvent extracts. AE, Aqueous Extract; EE, Ethanolic Extract and HE, Hexanic Extract

| CMI (µg/ml) | Safflower | Clove | Turmeric | Orange peel | Cubeb | Ginger root | | Red pepper | Mint | Miswak |
|---|---|---|---|---|---|---|---|---|---|---|
| Bacterial strains | HE | HE | EE | EE | EE | AE | HE | HE | HE | HE |
| **Gram (-)** | | | | | | | | | | |
| *E. coli* | na | na | na | na | na | na | na | na | na | 125 |
| *E. cloacae* | 250 | 125 | na | na | na | na | na | na | na | 125 |
| *K. pneumoniae* | na | na | na | 250 | na | na | na | na | na | 62.5 |
| *S. typhimurium* | na | 250 | na | na | na | na | na | na | na | na |
| **Gram (+)** | | | | | | | | | | |
| *B. flavum* | na | na | na | na | na | na | na | na | na | 125 |
| *S. xylosus* | 250 | 31.25 | 62.5 | na | 125 | na | 125 | 250 | 125 | 15.62 |
| *S. aureus* | na | na | na | na | na | na | na | na | na | 15.62 |
| *S. epidermidis* | na | na | na | na | na | na | na | na | na | 31.25 |

na, not active (MIC > 1000 µg/mL)

Cinnamon and clove ethanolic extracts displayed the highest values (196.57 ± 2.23 and 194.06 ± 5.56 µg GAE/mg, respectively) followed by the aqueous extract of black tea (198.16 ± 5.90 µg GAE/mg). These results were in agreement with those of Shan et al. (2005) and Dragland et al. (2003) who compared the phenolic contents of methanolic and boiling-water extracts of various spices and medicinal plants from Hong Kong and Norway, respectively. The authors have reported that clove and cinnamon extracts contained the highest level of phenolic among all the plant materials tested. For the hexanic extracts, that of corchorus showed the highest level of phenolic content with a value of 130.89 ± 1.30 µg GAE/mg.

The total flavonoid contents of extracts varied from 1.65 µg QE/mg (water extract of red pepper) to 340.13 µg QE/mg (ethanolic extract of miswak) (Figure 2). In general, high levels of flavonoid were found in hexanic extracts followed by ethanolic then aqueous ones. Results clearly show that flavonoid contents increase when decreasing the solvent polarity, as it was reported previously (Zarai *et al,* 2013). The use of several solvents with different polarities allows to separate flavonoid based on their degrees of glycosylation, acylation or methylation (Welch *et al,* 2008).

*3.2. Radical scavenging activity*

A large number of methods have been developed to evaluate total antioxidant capacity of food and dietary supplements, herbal extracts or pure compounds. Assays based on the use of DPPH radical scavenging activity are among the most popular spectrophotometric methods for determination of the antioxidant capacity. This procedure is easy, rapid, sensitive and reproducible (Ozcelik *et al,* 2003). It is based on the reduction of alcoholic DPPH solution in the presence of hydrogen-donating antioxidants due to the formation of non-radical DPPH-H form (Koleva *et al,* 2002; Pourmorad *et al,* 2006; Lin *et al,* 2009). Table 2 illustrates the radical scavenging activity of extracts expressed in terms of $IC_{50}$. Smaller values of $IC_{50}$ correspond to higher antioxidant capacities. The BHT was used as reference for radical scavengers with an $IC_{50}$ of 5.4±1.25 µg/ml. The $IC_{50}$ of the different extracts ranged from 1.45 µg/ml for alcoholic extract of cinnamon to 1224.89 µg/ml for hexanic extract of coffee (Table 2). Also it was observed that ethanolic and aqueous extracts exhibited strong antioxidant activities. However, hexanic extracts showed weak activities except for miswak, cubeb and mint which exhibited a high radical scavenging activity with $IC_{50}$ value of 6.87, 13.35 and 16.6 µg/ml, respectively. Ramalakshmi et al. (2008) had described similar results when using different extracts from coffee beans using various solvents. They showed that polar solvent (methanol extract) displayed the highest antioxidant activity followed by the acetone, the chloroform then the hexane extracts which showed a very poor scavenging activity. Likewise, Anagnostopoulou et al. (2006) had reported that the aqueous extracts of sweet orange peel exhibited the strongest radical scavenging activity among various extracts tested.

The highest radical scavenging activity of aqueous extracts corresponded to those of cinnamon and coffee, with $IC_{50}$ values of 1.96 and 3.02 µg/ml, respectively. These activities were higher than that of BHT (Butylated hydroxytoluene). The strongest radical scavenging activities of aqueous extracts decreased in the following order: cinnamon > coffee > BHT ≈ cubeb > black tea > mint > fennel. In the case of ethanolic extracts, cinnamon, clove and black tea showed the highest antioxidant activity with $IC_{50}$ value of 1.45, 2.16 and 4.34 µg/ml, respectively. These activities were higher than that of BHT. The strongest antioxidant activities decreased in the following

order: cinnamon > clove > black tea ≈ BHT > bay laurel ≈ miswak > common vervain > turmeric > green tea.

Previous studies reported that spices belonging to the Umbelliferae (Fennel, caraway, cumin, coriander, anise) and to the Piperaceae (black peper, cubeb) families possess strong antioxidant activities (Zarai *et al,* 2013; Melo *et al,* 2005; Singh *et al,* 2004; Duthie *et al,* 1999). However, no comparison was made with spices from other families. In the present study, we found that all tested spices of the Umbelliferae (Fennel, caraway, cumin, coriander, anise) and Piperaceae (black peper, cubeb) families displayed weaker antioxidant activities than those from Lauraceae (cinnamon, bay laurel), Myrtaceae (Clove), Labiatae (Mint) and Theaceae (Tea) families. In particular, cinnamon aqueous and alcoholic extracts exhibited the strongest antioxidant activity among all extracts tested.

Phenolic compounds are antioxidants which act as free radical terminators (Shahidi and Wanasundara, 1992). The statistical analysis carried out on all extracts showed that there was no correlation between antioxidant activity and total phenolic and flavonoid contents ($R^2 < 0.1$). Such a correlation was reported by some studies (Maillard and Berset, 1995; Heinonen *et al,* 1998; Kahkonen *et al,* 1999; Oktay *et al,* 2003; Shan *et al,* 2005; Pourmorad *et al,* 2006) and denied by others (Bocco *et al,* 1998; Czapecka *et al,* 2005; Wong *et al,* 2006; Ebrahimzadeh *et al,* 2008). The absence of correlation might be explained by the fact that other compounds display also an antioxidant activity. In fact, other antioxidants such as vitamins (A, C,and E), minerals (Selenium), carotenoids (betacarotene, lycopene) and polyphenols were described in foods (Heinonen *et al,* 1998; Bartolome *et al,* 2004). As it was postulated before (Shan *et al,* 2005), we think that discrepancies between results from many studies are likely to be due to the genotypic and environmental differences within species, the choice of parts tested, the harvesting method and the antioxidant test used.

### *3.3. Antibacterial activity*

The antibacterial activity of solvent extracts was evaluated against Gram-positive (*B. flavum*, *S. xylosus*, *S. aureus* and *S. epidermidis*) and Gram-negative (*E. coli*, *E. cloacae*, *K. pneumoniae*, *S. typhimurium*) strains associated to several human diseases. The activity was assessed by measuring the inhibition zone diameter (Table 3) and by determining the minimum inhibitory concentration (MIC) values (Table 4). Ampicillin was used as a positive control. Extracts displayed antibacterial activities against most of the bacteria tested. However, these activities were significantly lower than that of ampicillin. This is probably due to the fact these extracts are crude preparations. Further purifications might be needed to yield more active compounds (Fabry *et al,* 1998). Aqueous extracts showed a weak inhibitory activity against all strains tested except for ginger root extract which exhibited a high activity against *K. pneumonia*. Likewise, the alcoholic extracts showed low inhibitory activities against all bacterial strains tested except ginger root and orange peel extracts against *K. pneumonia* and turmeric extract against *S. xylosus* bacteria. The hexanic extract displayed a broad antibacterial spectrum and exerted significant antibacterial effect against both Gram-positive and Gram-negative bacteria tested. *S. xylosus* was found to be highly sensitive to most of the hexanic extracts tested and especially to those of red and chili pepper, clove, ginger root, mint, miswak and safflower. These findings were in line with reports that hexanic extract of *Diospyros canaliculata* showed a strong antibacterial activity against all tested pathogenic agents compared to the aqueous, ethyl acetate and methanolic extracts (Kuete *et al,* 2004). Likewise, Çetin et al. (2010) showed that the essential oil and the hexanic extract of *Foeniculum vulgare* were effective against most of the foodborne pathogenic, saprophytic, probiotic, and mycotoxigenic microorganisms tested. Moreover, Yukiko et al. (2002) and Soharb et al. (2001) reported that hexanic extract of *Prangos pabularia* and *Claussena heptaphylla* exhibited a strong antibacterial activity due to the presence of a large amount of volatile compounds known to be effective on most of pathogenic strains.

Table 4 shows the minimum inhibitory concentration (MIC) (µg/mL soluble extract) values of plant extracts displaying a high antibacterial activity against different strains tested. The MIC values varied from 15.62 to 1000 µg/mL (Table 4). Hexanic extracts of miswak, clove and turmeric showed high action against *Staphylococcus* strains with MIC values of 15.62 and 31.25 and 62.5 µg/mL, respectively. Likewise, Chentouf et al. (2012) described a strong antibacterial activity of miswak extract on *Staphylococcus* strains. In addition, Al Lafi and Ababneh (1995) reported also a strong antimicrobial effect of miswak derivatives against *Streptococcus sp.* and *S. aureus*. Keskin and Toroglu (2011) have studied antimicrobial activities of ethyl acetate, acetonic and methanolic extracts of 12 plant species. The authors demonstrated that methanolic and acetonic extracts of clove exhibited the highest antibacterial activities against *K. pneumonia*, *B. megaterium*, *P. aeroginosa*, *S. aureus*, *E. coli*, *E. cloaca*, *C. xerosis* and *S. faecalis*.

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