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Volatile Constituent of Cinnamon and Their Potential Antioxidant and Antimicrobial Activities

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ABSTRACT

In the present work, the volatile compounds of Cinnamomum zeylanicum were detected and identified by GC–MS and FTIR analysis. GC–MS allowed us to identify 60 compounds and indicated that the main compounds constituting the volatile oil were mainly Cinnamic aldehyde (81.52%), Eugénol (2.91%), p-Cineole (2.91%), Camphene (2.12%), α-pipene (1.48%), Hydrocinnamic aldehyde (1.21%) and α-Terpineol (1.04%) this compounds were also identified by FTIR analysis. The essential oil was also subjected to a biological screening for its possible antioxidant activities by means of DPPH radical scavenging test, the sample tested showed slight antioxidant activity in comparison with the positive control (Ascorbic acid). Cinnamomum zeylanicum essential oil was examined also against a panel of 10 bacterial strains using the agar diffusion method. The obtained results have shown that the essential oil exhibited moderate to strong activity against the tested microorganisms. This results suggested that the Cinnamomum zeylanicum essential oil possesses a good antimicrobial and antioxidant properties, and is a potential source of active ingredients for food and pharmaceutical industry.

Keywords: *Cinnamomum zeylanicum*, Essential oil, Chemical Composition Antimicrobial Activity, Antioxidant Activity.

1. INTRODUCTION

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, phytotherapy, spices and nutrition [1]. Because of the large number of its properties, Cinnamon is indicated in a number of pathologies or of organic and functional disorders. Internally, it is indicated in asthenia, fever aches, flu, infections due to cooling and syncope. It is also used in intestinal disorders of any kind such as: intestinal infections (cholera), gastric atony, slow digestion, intestinal parasites, digestive spasms, spastic colitis and diarrhea. It may also be indicated in the bleeding, vaginal discharge, hemoptysis and impotence. It was formerly very recommended cold seasons melancholic, with insufficient digestive, the elderly. Externally, Cinnamon is used to combat lice, scabies and treat wasp stings and snakebites [2].

2. MATERIALS AND METHODS

2.1. Plant Material

For the IX th edition of the French Pharmacopoeia, the drug is made by dry aromatic yellowish brown barks and whose taste is sweet and pungent. Our samples come: from among herbalists [3].

2.2. Isolation of the Essential Oil

Obtaining essential oil was carried out in a Clevenger-type apparatus [4]. A steam distillation was performed by boiling for an hour and a half to 200 g of plant material with one liter of water in a two liter flask surmounted by a column of 60 cm length connected to a condenser. The yield of essential oil is expressed as the volume of oil (in ml) obtained for the mass of 100 g dry plant material.

2.3. Chromatography Analysis

The GC-MS analysis was performed using a Hewlett Packard 5973-6800 system operating in EI mode (70 eV) equipped with a split/splitless injector (250°C), a split ratio 1/50, using a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 µm. The temperature program for the HP-5 MS column was from 60°C to 280°C at a rate of 2°C/min. Helium was used as a carrier gas at a flow rate of 0.5 ml/min. Injection volume of the sample was 0.2 µl. The identification of the components was conducted in an IS system managing a library of spectrum wiley7n.l.

The GC-MS analysis was performed at the Scientific and Technological Scientific Research Center on Physico-Chemical Analysis (CRAPC), Bab ezzouar (Algiers, Algeria).

2.4. FTIR Analysis

The Fourier transformed infrared spectroscopy (FTIR) is realised using a Perkin Elmer apparatus (Universal ATR Sampling Accessory), the range of analysis was 4000-600 cm-1.

Results are compared with the internal Euclidean bibliography of the device. The FTIR analysis was performed at the Regional Police Scientific Laboratory (Constantine, Algeria).

2.5. Antioxidant Activity

The antioxidant activity of Cinnamon oil was measured in terms of hydrogen-donating or radical-scavenging ability, using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent Burits and Bucar [5]. Fifty microliters of each concentration (200, 400, 600, 800 et 1000 µg/ml) of each sample was diluted in methanol and were added to 5 ml of methanolic solution of DPPH (0,004 %). The inhibition of the DPPH by the ascorbic acid was even analyzed at the same concentration for comparison. Absorbance measurements were read at 517 nm, after 20 min of incubation time at room temperature. Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the negative control. All determinations were performed in triplicate. The inhibition percentage of the DPPH radical by the samples was calculated according to the formula presented by Sharififar et *al*. [6]:

$$\% \text{ inhibition} = ([A_{White} - A_{Sample}]/A_{White})$$

where:

A_{White}: the absorption of the blank sample (t = 0 min)

A_{Sample}: the absorption of the tested oil or substance solution (t = 20 min).

The kinetic reactions of the essential oil and the ascorbic acid with DPPH, were mentioned for every concentration tested. The essential oil and the ascorbic acid of the inhibited DPPH, were recognized at the end of the reaction in order to reach the index IC_{50}. This parameter is defined as the antioxidant concentration required to reduce the concentration of the initial DPPH of 50 % [6].

2.5.1. Time balance Determination of TEC_{50}

TEC_{50} parameter was defined when time reached balance with an antioxidant concentration equal to IC_{50}. This time is graphically calculated.

2.5.2. Determination of the Antiradical Efficiency (AE)

The two factors IC_{50} and TEC_{50} were combined in order to get the Anti-radical efficiency:

$$AE = AA/IC_{50} \times TEC_{50}$$

2.6. Statistics Analysis

The classical methods of statistics were used to calculate the average and the standards deviations. All measurements were performed in triplicate, and results were presented as an average ± standard deviation. Analyses of variation were realized by ANOVA with the software «SPSS». The probability of *p* inferior to 0.05 was admitted as a criterion of a significant difference.

2.7. Antimicrobial Activity

In this study 10 different bacteria were used. The bacterial group included 06 Gram-negative bacteria, namely *Escherichia coli*, *Shigella*, *Proteus mirabilis*, *Enterobacter aerogenes*, *klebsiella oxy*, *Klebsiella pneumoniae*, and 04 Gram-positive bacteria namely *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilus*, *Enterobacter faecalis*. All microorganisms were obtained from the Bacteriology Laboratoy, Faculty of Medicine, HCU of Dorban in Annaba (Algeria).

The antibacterial activities were carried out using the disc diffusion method on solid medium; the strains were reactivated using a 20 h culture growth at 37 °C and adjusted to 10^8 CFU/mL. The bacterial strains are sowed on the surface of the agar in radial spots form by means of swab and suspensions of young bacterial cultures prepared according to the CLSI (committee for laboratory standards institute [7]). The application is made by sterile filters paper disks (6 mm diameter, 06/limp) which were placed on the inoculated agar surfaces and impregnated with 10 µL of the solution; the plates were incubated during 24 h at 37 °C [8]. The reading of the results is made by the measurement of the inhibition diameter around the disk.

3. RESULTS AND DISCUSSION

3.1. Essential Oil Yield and Chemical Composition

The quantitative analysis allowed us to get through hydrodistilation yield 1.1% essential oil.

The cinnamon bark contains 1-2% of essential oil is greater than that of water, freshly distilled pale yellow density, found in gasoline: 70% cinnamic aldehyde together with a little and eugenol various terpene hydrocarbons [9].

Moreover, according Khanfri, 2012 [10]: these results show that the yield of essential oil obtained by steam distillation of between 1.2% and 1.5% with a period exceeding 5 hours.

The physico-chemical properties have been measured as follows optical density: 1.050 [11] and refractive index: 1,6020 [12].

Early research on the composition of the essential oil from the bark of the Ceylon cinnamon were made by Blanchet in 1833.

Sometime later, Dumas and Peligot (1834 and 1835) indicate that the main component of this species is the "Cinnamaldehyde" or "cinnamic aldehyde" (Guenther, 1977) and (Vernon and Richard, 1976).

Other compounds were identified in 1892 and 1902 by chemists of Schimmel, in particular, Walbaum and Hüthing, who note that the content of eugenol in the essential oil of the leaves is higher than that of the cortex (Guenther, 1977), and they demonstrated several compounds by preparation of chemical derivatives (chemical reactions) (Vernon and Richard, 1976).

On the other hand, Valnet 1990, determined that bark Cannellier Ceylon (Cinnamomum zeylanicum) is very high in essential oil, 1-pinene, in cineol, in phellandrene, in furfural, in cymene, linalool, sugar mucilage, tannin, starch, mannitol ... the bark's essential oil itself is composed of 65 to 75% of cinnamic aldehyde, 4 to 10% eugenol, carbides and terpenic alcohols.

According to Wright, 1995 approximate chemical composition of the essential oil of cinnamon bark: Cinnamaldehyde (trans form) (76%), eugenol (4%), cinnamyl acetate (5%), l-linalool (2%), β-caryophyllene (3%), α-terpineol (0.7%), Coumarin (0.7%), 1,8-cineole (0.6%), Terpinene-4-ol (0.4%) [13].

Our results on the chemical profile of essential oil of the *Cinnamomum zeylanicum* matches those of (Khanfri, 2012) [10], which showed that the essential oil cinnamon analyzed by GC-MS revealed the presence of 37 dominant components are characterized by the high concentration of: E-cinnamaldehyde (63.23%), camphene (19.74%), a-pinene (14.53%) and 1,8-cineole (6.52%).

Table 1. Chemical composition of *Cinnamomum zeylanicum* essential oil.

N	Compounds	Retention time (min)	(m/m) %	N	Compounds	Retention time (min)	(m/m) %
1	Cyclopropylmethanol	4.854	0.007	34	Trans-2-Decenal	32.843	0.006
2	Styrene	7.997	0.051	35	**Cinnamic aldehyde**	36.418	**81.529**
3	Pinene	9.531	0.017	36	3-Phenylpropenal	36.930	0.039
4	Bicyclo[3.1.0]hexane	9.814	0.038	37	Styrone	37.061	0.092
5	**α.-Pipene**	10.252	**1.482**	38	Linarodin	37.344	0.099
6	Camphene	11.061	0.741	39	3-Phenylacrolein	38.781	0.013
7	Benzaldehyde	11.860	0.537	40	α.-Copaene	40.408	0.184
8	Pseu dopinene	12.766	0.516	41	Bergamotene	42.888	0.021
9	β.-Pinene	13.745	0.017	42	β.-Caryophyllen	43.083	0.084
10	Cymene	15.982	0.159	43	Cumarine	44.837	0.569
11	**p-Cineole**	16.513	**2.914**	44	**Camphene**	45.300	**2.124**
12	Salicylal	17.326	0.035	45	**Eugenol**	45.494	**2.915**
13	β.- Ocimene	17.672	0.010	46	α.-Longipinene	46.644	0.016
14	delta.-3-carene	18.335	0.073	47	α.-MuuroleneBenzyl	48.072	0.035
15	Hypnone	18.978	0.024	48	Benzyl phthalate	48.768	0.032
16	α.-Terpinen	20.410	0.038	49	Delta-Cadinene	49.441	0.100
17	Terpinolene	21.526	0.211	50	3-Ethylquinoline	50.620	0.013
18	Fenchyl alcohol	22.427	0.029	51	α.-Calacorene	52.719	0.009
19	3-Chromene	24.346	0.076	52	Caryophyllene oxide	52.919	0.106
20	Linalol	24.741	0.044	53	Anthracenedimethanol	53.138	0.009
21	Isoborneol	25.477	0.018	54	Naphthalene	55.506	0.101
22	**Hydrocinnamic aldehyde**	26.066	**1.218**	55	Cedreanol, (-)-	56.310	0.076
23	2-Bornanol (CAS)	26.275	0.474	56	Delta-Cadinene	56.548	0.025
24	1,2-Chromene	26.607	0.160	57	β.-Gurjunene	56.992	0.025
25	4-Carvomenthenol	26.996	0.773	58	Hydroxycarbostyril	57.333	0.022
26	p-Cymen-8-ol	27.791	0.009	59	4-Phenox yacetophenone	58.025	0.023
27	**(-)-.α.-Terpineol**	28.088	**1.041**	60	Bisoflex 81	97.330	0.060
28	Estragol	28.414	0.022				
29	Cinnamal	29.949	0.705				
30	Allylbenzol	31.108	0.007				
31	p-Isopropylben zaldehyde	31.240	0.072				
32	Phenylsulfonyl	31.357	0.006				
33	l-Carvone	31.537	0.147				

3.2. Antioxydant Activity

The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen-donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [14]. The scavenging ability of the essential oil and positive control are presented in Table 2.

Table 2. DPPH scavenging activity (%) of essential oil and standard antioxidant.

Concentrations (µg/ ml)	Essential oil	Ascorbic acid
200	02,65±0,009	26,20±0 ,077
400	03,67±0,011	32,65±0 ,098
600	03,80±0,013	51,89±0 ,155
800	03,92±0,011	72,15±0 ,218
1000	04,92±0,014	94,81±0 ,286

3.3. Kinetic Reaction

The kinetic reactions of the free radical DPPH obtained for each concentration of the ascorbic acid and of the essential oil are mentioned in Figure 1 and 2.

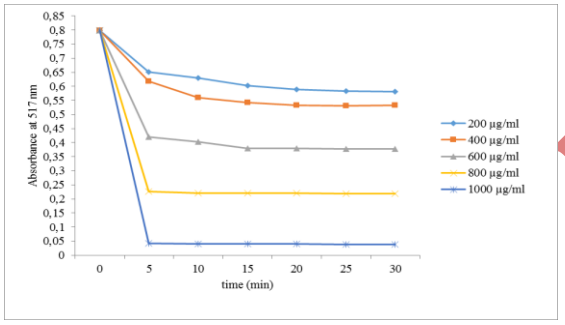

Figure 1: Kinetic reduction of DPPH of ascorbic acid.

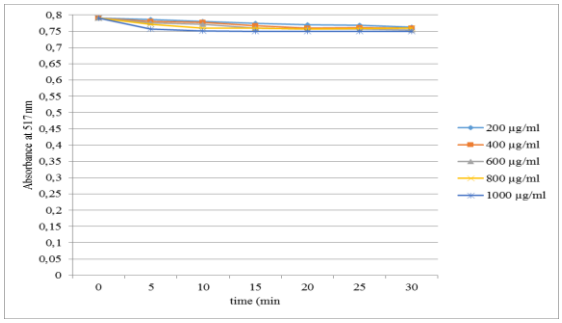

Figure 2: Kinetic reduction of DPPH of essential oil.

From the results obtained, we found that the biphasic reaction has a quick weakening of absorbance during the first minutes, followed by a slow step till balance is reached, so we can distinguish two areas: area of strong kinetic of DPPH radical scavenging with radical scavenging absorbed during the first 5 minutes, as for the ascorbic acid for all concentrations during ten minutes for a concentration of 1000 µg/ml. This area is observed during the first fifteen minutes for the essential oil. A second area with a slow kinetic of DPPH radical scavenging a tendency zone towards a recognized balance after five minutes for all concentrations of the ascorbic acid except the concentration 1000 µg/ml. For the essential oil of this area, it is recognized after fifteen minutes.

While making the reaction between DPPH and the ascorbic acid with hydrogen, we can recognize in this reaction that balance is reached in a short period of time compared to the essential oils. The antioxidant activity is dependent to the hydrogen atom movement of the hydroxyl group of the phenolic components of the essential oil. In presence of the free radical DPPH, the H atom is transferred to DPPH stable molecule. This induce a diminution in the concentration of the free radical and the absorbance during reaction till the weakening of the antioxidant capacity as a hydrogen donor.

The inhibition percentage results of the radical DPPH are mentioned in Figure 3.

We observe that the inhibition percentage of the free radical of the essential oil is low to those of the ascorbic acid for all concentrations used.

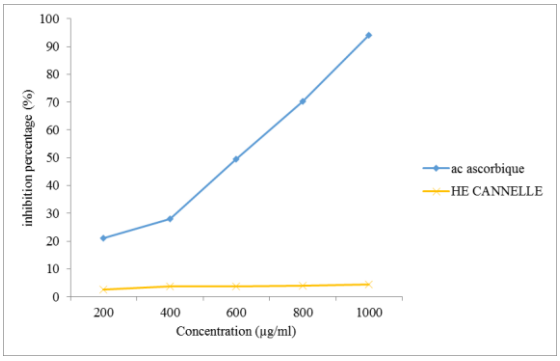

Figure 3: The inhibition percentage of essential oil and ascorbic acid.

We observe that the inhibition percentage of the free radical of the essential oil is low in comparison to those of the ascorbic acid for all concentrations used. For the highest concentration (1000 µg/ml), the essential oil revealed an inhibition percentage of 04,92 %, while the ascorbic acid is inhibited with 94.18 % of DPPH [15].

3.4. IC_{50} Determination

The IC_{50} is the quantity of the antioxidant needed to reduce the concentration of the free radical DPPH to 50%. We have chosen the state of balance as a period of measurement where growth reaction can't go further. Timing of balance state depends on the reactivity of the essential oil and the concentrations used. We recognize that the ascorbic acid reacts rapidly with DPPH. The IC_{50} for essential oil studied was 15±1 min, so the ascorbic acid needs just 5±0,66 min to reduce the concentration of the radical free to 50%. It`s well known that not only the main components of the essential oil are responsible for the antioxidant activity, this activity may be attributed also to the minor components that may interfere in synergy and antagony to create this system against the free radicals [16,17].

3.5. Antimicrobial Activity

The microbiological study of the essential oil of pure cinnamon with dilution technique in a solid medium (agar) on the growth of bacteria; all microbiological results obtained during the study shows that all the products tested have a very significant antibacterial activity, in which some strains seem to be distinguished by a very high sensitivity (Khanfri, 2012) [10]. Cinnamaldehyde would also include compounds providing cinnamon antimicrobial properties[18].

In fact, for ages, spices like cinnamon are used to extend the shelf life of food. Studies on cinnamon extracts showed today that it can help reduce the multiplication of several microorganisms [18,19].

Table 3. Results of the antibacterial activity.

Microorganisms	Sensitivity*
E. coli	+++
S. aureus	+++
M. luteus	+++
B. subtilus	+++
Shigella sp	+++
P. mirabilis	+++
E. faecalis	+++
E. aerogenes	+++
k. oxy	+++
K. pneumanae	+++

Each value represents the mean of two replicates ± standard deviation

*The sensitivity to the different strains was classified by the diameter of the inhibition zone as follows [19]:

-: diameter less than 8 mm, not sensitive;

+: sensitive, diameter 9-14 mm;

++: very sensitive, diameter 15-19 mm;

+++: extremely sensitive for diameter larger than 20 mm.

4. CONCLUSION

In the present work, we have characterized the chemical composition of *Cinnamomum zeylanicum* essential oil. The identification of the chemical constituents was realized on the basis of FTIR and GC-MS analysis.

The FTIR analysis permitted the qualitative identification of ten (10) components, in the other hand the chromatographic analysis permitted the qualitative and the quantitative identification of 60 components with the co-dominance of the Cinnamic aldehyde (81.52%), Eugenol (2.91%), p-Cineole (2.91%), Camphene (2.12%), α-pipene (1.48%), Hydrocinnamic aldehyde (1.21%) and α-Terpineol (1.04%). The antimicrobial potential of *Cinnamomum zeylanicum* essential oil was evaluated against pathogenic bacteria stains. The use of the volatile formulations on the basis of medicinal and aromatic plants may present several advantages for today` synthesis products. In fact; the essential oils are less toxic for the environment and may have a high biocide activity. Our interest was at the same time for the antioxidant activity of the essence of the *Cinnamomum zeylanicum* for the purpose to find new natural antioxidant in order to avoid the use of synthetically ones which may some of them be toxic or carcinogenic.

The Results obtained confirm that the medium antioxidant potential of the essential oil of this plant according to others. This antimicrobial and antioxidant activity is due mainly to its fullness of Cinnamic aldehyde. These results keep an open perspective for research of formulations on the basis of essences of the *Cinnamomum zeylanicum* in place of other synthesis preservatives or antioxidant on the basis of plant used in the field of food industry, pharmaceutical and cosmetics industry.

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