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Curcuma Longa L. *Specie Ecological, Adaptation Conditions and Biological Trials* †

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Abstract: Interest on *Curcuma Longa* L specie also called "Golden spice" is increasing due to its several applications in different fields: culinary, pharmaceutics, nutraceutics, cosmetics, agro-industry... Thus, the present work deals with ecological conditions for the adaptation of such specie, qualitative and quantitative phytochemical study and biological applications as in vitro antioxidant and antibacterial activities. Investigations on ecological conditions: soil and climate; exhibit and a possible adaptation of studied specie in North-eastern Mediterranean border of Algeria. Qualitative analysis of the secondary metabolites contained in *Curcuma longa* L. rhizomes extract was carried out by chromatography on a thin layer TLC, physical and chemical revelation exhibit a strong presence of polyphenols. Quantification of the polyphenols and flavonoids was carried out by UV spectrophotometry, which revealed a content of 18.125 mg/g EAG for polyphenols and 5.718 mg/g EQ for flavonoids. Evaluation of the antioxidant power was carried out using the DPPH free radical scavenging test, showed a strong antioxidant activity (99%) greater than used standards ascorbic acid (96%) at the same concentrations, this activity is correlated to a richness in phenols content which make it among the most powerful antioxidants in nature. Antimicrobial activity was carried out by the disk diffusion method, obtained results showed an efficacy against most of tested strains even at very low doses. According to the encouraging obtained results, it would be interesting to try implantation of this specie by local start-ups and enhance it by extending the range of applications in order to isolate, purify and identify the compounds present in the extract and identify the active compound(s) responsible of this effect to develop new drugs and face the phenomenon of germs resistance to antibiotics and find new efficient antioxidant agents.

Keywords: *Curcuma Longa* L; eco-adaptation; secondary metabolites; antioxidant; antimicrobial activity

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1. Introduction

Turmeric was widely used since long centuries in traditional medicine [1,2]. Nowadays, scientific researches proved that the main constituent of *Curcuma* species "Curcumin" is responsible of the biological activity of turmeric such as anti-inflammatory, antimicrobial, anti-oxidant, anti-parasitic, anti-mutagenic, anti-cancer and anti-cardiovascular diseases [3–6].

These properties have been attracting for a long time the interest of various scientists, since this natural product with multi-targeted effects have less adverse effects than synthetic drugs with non observed toxicity even when taken at very high dose over long time [7,8], however these therapeutically effects are limited by a luck of solubility due to its

diketo chemical structure, and one way of recent researches for improving its physico-chemical proprieties and reactivity, is structural modulation and transformation, another way is to find an appropriate medium to generate a keto-enol tautomeric form and stabilize it.

Therefore, to meet industry demands for these biobased compounds with high quality, extraction is by far the most important stage in the recovery process, and depending on different factors such as high productivity, integrity and selectivity towards the target compound must be considered [9]. Extraction efficiency also depends on the localization and nature of the polyphenols.

In this context, the present study reports investigations on Turmeric specie culture's ecological conditions, Soxhlet extraction, Bioactive compounds quantification and in vitro assessment of its antioxidant and antimicrobial activities. Furthermore, extraction, isolation, purification and identification of main curcuminoids are given, in order to promote its introduction and exploitation by young start-ups.

2. Experiments

2.1. Plant Materials

Turmeric rhizomes were commercially purchased from a local traditional shop, cleaned, dried and sprayed into a powder with an electrical pulvirisator until content was about 100 g dry weight. All other solvents and reagents used in this work were of analytical grade, and strains ATCC referenced.

2.2. Ecological Culture Conditions

Investigations on eco-conditions of *Curcuma longa* L. specie culture includes: temperature, soil, brightness, humidity, rainfall, altitude.

2.3. Extraction

Solvent extraction was carried out using a Soxhlet.

Briefly, it consisted into the extraction of the hole crud of rhizomes (76.23 g) with 70 mL of Ethanol, for 3 h, and then filtered with a Büchner leading to crud extract, the filtrates were evaporated to concentrates using rotary evaporation at 40 °C and stored in an amber coated bottle at 4 °C. The extraction yield was calculated as follows:

Yields are calculated according to the following formula:

$$\text{Yield \%} = (\text{Crude extract mass/powder mass}) * 100 \quad (1)$$

2.4. Total Polyphenols Content

0.2 mL of each sample was firstly mixed with 1 mL of diluted Folin–Ciocalteu reagent (5/10 H_2O) by vortexing. After that, 0.75 mL of Na_2CO_3 (7, 5%) are added. Then, the reaction mixtures are further incubated for 2 h at room temperature in the dark, and finally, the absorbed optical density is recorded at the wavelength of 765 nm [10,11].

2.5. Total Flavonoid Content

0, 4 mL of diluted sample with 1 mL ethanol is separately mixed with 1 mL of 2% aluminum chloride methanol solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture is measured at 430 nm with spectrophotometer [12].

2.6. The Antioxidant Activity Analysis

The DPPH scavenging activity was evaluated according to Blois method [13]. 160 µL of DPPH methanol solution (6 mg/100 mL) were added to 40 µL of extracts, the mixture was then incubated for 30 min in the dark at room temperature. The absorbance was measured at 517 nm. A blank of 40 µL of methanol with 5 mL of DPPH solution was used as negative control. α-tocophérol, BHT and BHA were used as positive controls.

2.7. The Antimicrobial Activity Analysis

The antimicrobial susceptibility and resistance tests of our extracts were carried out according to the Agar disk-diffusion testing developed in 1940 [14].

Discs (Whatman No. 1, 6 mm diameter) are impregnated with each extract and then applied to the surface of the agar plates which have been seeded by spreading the microbial suspension. The seeding is carried out in such a way to ensure a homogeneous distribution of the bacteria. The petri dishes are incubated during 24 h at the appropriate temperature 37 °C in the laboratory oven, and the resulting inhibition zone diameter was measured in millimeters using a ruler.

Antimicrobial activity is determined in terms of the diameter of the inhibition zone produced around the discs.

3. Isolation of Curcuminoids

3.1. Thin Layer Chromatography

A rapid screening of suitable solvent system for isolating maximum compounds of turmeric rhizomes extract was done using thin layer chromatography (TLC). Physicochemical revelation of spots was done using U.V lamp at 254 and 365 nm, R*f* values were then calculated according to Touchstone [15].

3.2. Column Chromatography

Isolation of curcuminoids from Turmeric was done through column chromatography using the mobile phase selected on the basis of TLC screening. A glass column (100 × 3 cm) was filled with silica gel powder Kieselgel Merck (60–120 mesh), and was then loaded with hexane. Afterwards, concentrate crude extract was added on top with a pipette. The compounds of rhizomes extract were isolated using a gradient of 0, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100% ethyl acetate in hexane as mobile phase, with a drip elution rate. Afterward, these isolated fractions were analyzed through TLC again. The isolated fractions containing the same compounds based on R*f* values were pooled together, and fractions having a single spot on TLC plates were subjected to characterization and identification analysis.

3.3. ^{1}H-NMR Identification

1D-1H-nuclear magnetic resonance (NMR) spectrum of the isolated and purified compounds was performed using NMR (Bruker Avance), 400 MHz, in $CdCl_3$, 1H chemicals shifts (δ) are given in ppm (the residual peak of deuterated solvent was used as reference).

3.4. Statistical Analysis

Each experiment was performed in five replicates and the data was subjected to calculations of mean ± S.E. The mean values were used for drawing the graphs.

4. Results and Discussion

4.1. Ecological Culture Conditions

According to references http://climate-data.org/[16], recorded optimal culture conditions were for temperature (from June to October), a silica clay soil, soft brightness, high humidity, rainfall >900 mL, altitude 450–1200 m, Which correspond mostly to North-eastern Mediterranean border climate in Algeria, greenhouse and rational irrigation could also be considered in case of temperature and rainfall lack.

4.2. Total Phenol and Flavonoid Compound Content Results

The total phenol and flavonoids content shows (18.12 ± 0.01) μg EGA/mg DE and (5.718 ± 0.04) μg QE/mg of total polyphenols and flavonoids content respectively.

4.3. Evaluation of Biological Activities

4.3.1. Antioxidant Activity

The antioxidant capacity of *Curcuma longa* L. *rhizomes ethanol* extract was determined using DPPH free radical scavenging test potency with an IC50 = 86.4 ± 0.01 μg/mL, which is higher than referential used standard ascorbic acid (IC50 = 110 ± 0.00 μg/mL) and is in agreement with obtained total phenols content.

IC50% is defined as antioxidant concentration required to reduce 50% of initial free radicals concentration, and to better evaluate it two factors were calculated.

4.3.2. Antimicrobial Activity Results

• **Antibacterial test**

The diameters results of the growth inhibition zones exhibit an important antibacterial potential, at low concentrations, thus we noticed:

- A minimum inhibition concentration MIC of 7.81 ± 0.4μg/mL for *Bacillus subtilis and Escherichia coli.*

- And MIC =31.25 ± 0.9μg/mL for Staphylococcus aureus.

Imipenème IPM (10 μg/disc) and Nalidixic Na (30 μg/disc) were used as positive control.

• **Antifungal activity test**

Antifungal activity against *Trichoderma harzianum Rifai* reveal that there is no inhibiting potential against studied strain which is resistant to plant rhizomes extract even at high dose.

5. Isolation of Curcumin from Turmeric Rhizome Extract

Rhizomes' ethanol extract (12.31% of yield), was subjected to isolation of bioactive compounds through TLC and column chromatography. Hexane: ethyl acetate (70:30; *v*/*v*) solvent system gave a good resolution for isolating curcuminoids on TLC plate. The gradient solvent system used in column chromatography isolation lead to elute a total of 9 fractions of 50 mL each. Only F3, F4, F5 gave a single, yellow colored spot, whereas the other fractions gave multiple spots, F4 with a R*f* value of 0.78 corresponding to the main standard compound Curcumin, then was characterized using 1H-NMR spectroscopy.

Identification and Characterization of Curcumin through 1HNMR,

The 1H-NMR spectrum of the isolated compound F4 exhibit six peaks regions: C, H, D, E, F, G [15–17]**.**characteristic of Curcumin functions, Therefore, the isolated compound F4 was identified as [1,7-bis-(4-hydroxy-3-methoxyphenyl)-1,6-hepta diene-3,5-dione].

Although, Curcumin is commonly named as 1,7-bis (4-hydroxy-3-méthoxyphényl) -1,6-hépatadiène3,5-dione, highlighting its dikitone tautomeric form, however, few studies reported also two other possible rare forms asymmetric kito-enol tautomers attributed to its interactions with acid ph medium and solvents like CDCl3 [17,18].

Indeed, apparition of a doublet in region I (**6.4–6.5 ppm)** indicating the presence of non equivalent proton and the appearance of a $H_{hydroxyl}$ at **4.1 ppm** suggests the kito-enol forms of Curcumin.

The importance of the tautomerism of Curcumin has been examined in a study investigating the molecular mechanism of the observed synergy between Curcumin and water soluble antioxidants in cancer chemoprevention at physical conditions [19]. It has been observed that the Curcumin radical preferentially exists as a phenoxyl-type species, which is more hydrophilic than the keto form. Being more hydrophilic, it is preferentially moved to the external side of the cell membrane; this effect is probably responsible of Curcumin effectiveness as a scavenger of carcinogenic free radicals [20].

6. Conclusions

The present study highlighted a possible eco-adaptation of *Curcuma longa* L. specie in the North-eastern region of Algeria, it also reports its efficient extraction, qualitative and quantitative phytochemical study and biological applications as in vitro antioxidant and antibacterial activities, which revealed a very high antioxidant potential for DPPH radical scavenging test and antimicrobial activity against *Staphylococcus aureus* ATCC 25923; *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 2592 *and Rhizopus oryzae* M491890.1 referential strains, in addition to the isolation and identification of major component Curcumin.

Obtained results, encourage local young start-ups to invest this field from raw material production to transformed products commercialization: cosmetics, nutraceuticals, functional foods…

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