Extraction And Curcuminoids Activity From The Roots Of *Curcuma Longa* By Rslde Using The Naviglio Extractor

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Abstract

In the present study the extraction of curcuminoids was performed from *Curcuma longa* roots, focusing the interest on curcumin, the major phenolic component of the root that has been shown to have a high antioxidant activity. A cold extraction technique was employed as an alternative to other conventional solid-liquid extraction techniques such as maceration, infusion, percolation and extraction with Sohxlet which expose curcuminoids to light, heat and oxygen causing the degradation and thus limiting the effectiveness of the processes themselves. The Naviglio Extractor (NE) or rapid solid-fluid dynamic extraction (RSLDE) is an innovative technology of solid-liquid extraction that can extract in a short time, compared to other existing techniques, bioactive substances present in the solid matrix using water as solvent, at room temperature. Curcumin is a plant pigment of very bright yellow orange color and is referred to as the E100 in the list of accepted color additives in the food and pharmaceutical

sector being devoid of toxicity. In the Indian and Asian cuisine it is used to prepare the curry and various typical local sauces while in the Ayurvedic medicine for many centuries to treat a variety of ailments. Recent studies have shown that curcumin exerts anti-tumor effects for its ability to induce apoptosis in cancer cells without cytotoxic effects on healthy cells; in fact, it can interfere with the activity of the transcription factor □ and □-TNF (Tumor Necrosis Factor) which has been linked to a number of inflammatory diseases and cancer diseases.

Keywords: *Curcuma longa* roots, aroma, curcuminoids, anti-tumor effects, Naviglio extractor

Introduction

Curcuma longa is an herbaceous plant, perennial, rhizomatous of the *Zingiberaceae* family, originally from South-East Asia, known for centuries, as well as its use in food as a spice or natural dye, also for its healing properties, especially in traditional Indian medicine. India is today the largest producer and exporter country with about 80% of world production, which amounts to about 1,100.000 t/year (Prabhakaran Nair, 2013).

From the root and rhizome, the underground stem of the plant (Figure 1), a spice of a characteristic golden color is obtained due to the presence of a group of phenolic compounds, classified as curcuminoids in which multiple biological activities have been attributed including antioxidant, antibacterial, antiviral and anti-tumor (Shanmugam et al, 2014).

Fig. 1. Rhizome of *Curcuma longa*.

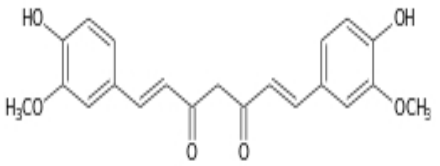


Fig. 2.Curcumin $C_{21}H_{20}O_6$

The crude extract of the dried and ground rhizome is used as a colorant in the kitchen and is cataloged as *Turmeric* and contains a mixture of curcuminoids, between 2-6%, responsible of the biological activity of the plant whose concentration varies depending on the species of Curcuma. The *Curcumin*, instead, is characterized by a mixture of three compounds, of which the main is curcumin (Figure 2), while the dimethoxycurcumin and the bis-dimethoxycurcumin which are respectively the mono and di-ester of methoxy curcumin (Sharma et al, 2013), representing 15-30% of the mixture.

In recent years, researchers have focused primarily on curcumin also known as diferuloylmethane, molecular formula ($C_{21}H_{20}O_6$), which is the

main biologically active component of turmeric, a phenolic compound lipophilic, poorly soluble in water and quite resistant to pH of stomach acid.

Our experimentation has been focused on the extraction of curcumin, from the roots of *Curcuma longa* by Naviglio extractor (Naviglio, 2003), which is a solid-liquid extraction system based on a negative pressure gradient which is provided in the two chambers of extraction and which allows to obtain in a short time an aqueous extract containing the curcuminoids. Subsequently, we proceeded to the quantification of the substances extracted by HPLC and curcumin obtained was compared with a commercial standard.

Materials and Methods

Reagents and Solvents:

All reagents and HPLC grade solvents were purchased from Merck (Darmstadt, Germany): anhydrous ethyl alcohol min. 99% (v/v); The water was produced with a Milli-Q Plus system (Millipore Corporation, Bedford, MA, USA). The standard of comparison (mixture of curcumin, and demethoxycurcumin bis-methoxycurcumin) was purchased from Sigma-Aldrich (Milan, Italy).

There are three Naviglio extractor models: the Lab model (capacity of 0.5, 1 and 2 liters); the Medium model (capacity 5, 10, 20 and 38 liters) and the model Industrial (capacity 50, 100 and 200 liters). In this work a Naviglio extractor (NE), Lab series of capacity of 0.5 liters (Atlas Filters Srl, Padua, Italy) was used for the extraction process. HPLC apparatus with 200 series pump (Perkin Elmer, Shellton, CT, USA), a UV/VIS Series 200 detector (Perkin Elmer, Shellton, CT, USA) set at 520 nm and a C18 column (250 x 4.6 mm particle size 5 □m) (Phenomenex, CA, USA).

Experimental part:

The ethanol/water extracts (50:50 v/v) were obtained in the following conditions:

20.0 g of *Curcuma longa* roots were extracted with 600 mL of an ethanol/water mixture (50:50 v/v) in the Naviglio extractor according to the method previously reported in the literature (Ferrara et al, 2002). Short static phase: 2 min; dynamic phase: 5 cycles with 12 sec. of the piston stop (2 min.); total cycles: 30 for a total of 2 hours.

After evaporation of the solvent, 375 mg of dry residue was obtained, which was dissolved in methanol and analyzed by HPLC for the analysis of bioactive compounds by means of a gradient eluent system. The eluents were: phase A: water and phase B: acetonitrile. The gradient applied was as follows: Time 0 min, 5% B; Time 3 min. 5% B; time 20 min. 100% B time 25 min 100% B. The flow was set to 1 ml/min, while the loop was 20 □L.

Results and Discussion:

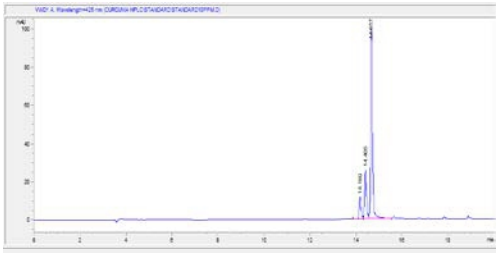
Figure 3 shows the HPLC analysis of the standard Curcumin with the three peaks related to curcumin, dimethoxycurcumin and bis-methoxycurcumin and Table 1 the relative retention times. Using scalar amounts of each standard calibration lines were constructed, in order to quantify the curcuminoids obtained from the extract of turmeric root by means of the Naviglio extractor.

Figure 4 shows the HPLC analysis of curcuminoids and Table 2 the relative retention times obtained by the Naviglio extractor using a solution ethanol/water (50:50 v/v). The use of ethanol was necessary to better solubilize the comminuted root of turmeric. The chromatographic profile revealed three separate peaks identified by comparison with standards. In particular, the peak 3 eluted at 14,237 minutes corresponds to bis-methoxycurcumin, the peak 4 eluted at 14,474 minutes corresponds to dimethoxycurcumin and the peak 5 eluted at 14,725 minutes corresponds to curcumin, which is the most abundant peak.

All determinations and the experiments were performed in triplicate and the results were reported as average value of 3 determinations. The standard deviation was calculated using Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA), and turns out to be less than 1%.

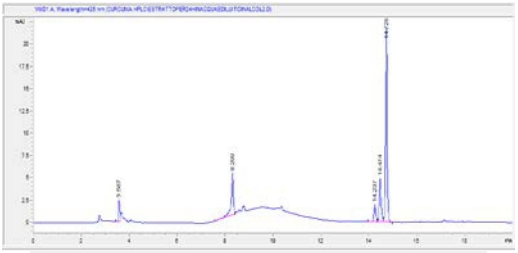
From the comparison of the chromatograms it is evident that in the hydroalcoholic extract in addition to curcumin the other unidentified curcuminoids were present, that are part of the *Turmeric* and are considered effective bioprotectors being able, unlike many other antioxidants, both to prevent the formation of free radicals, and to neutralize the existing free radicals (Krup et al,2013). In some studies, in fact, the powder obtained by grating the root of Curcuma was proven to be capable of inhibiting the growth of certain tumor cells *in vitro* and in a preclinical model of glioblastoma, for the synergistic effects of the mixture of all the bioactive compounds present (Zanotto et al, 2012).

Subsequently, by performing multiple chromatographic runs, it was possible to obtain the purified curcumin which was quantified using a calibration curve.



#	Time	Area	Height	Width	Symmetry
1	14.16	64.7	11.7	0.0821	0.666
2	14.405	136.6	26	0.0803	0.766
3	14.657	564.2	99.8	0.0849	0.705

Fig. 3. Chromatogram of standard Curcumin
Tab. 1 Retention times of standard



#	Time	Area	Height	Width	Symmetry
1	3.567	8	2.4	0.0498	0.827
2	8.299	29.9	4.7	0.09	1.858
3	14.237	10.9	1.9	0.0829	0.807
4	14.474	25.2	4.9	0.078	0.781
5	14.725	108.4	21.8	0.0769	0.841

Fig.4. Chromatogram of hydro alcoholic extract
Tab.2 Retention times of hydro alcohol extract
Naviglio Extractor
Naviglio Extractor

Pharmacological activities of curcumin:

In recent years, many studies and clinical trials carried out on the pharmacological activity of curcumin revealed its healing properties and its potential use as a nutraceutical for a large number of diseases (Kocaadam et al, 2015). Many of its activities, including the anticoagulant, antithrombotic, antihypertensive, anti-inflammatory, anti-diabetic, cholesterol lowering agents, antioxidant, hepatoprotective and antiviral were clinically proven.

Antioxidant property is higher than that of vitamin E, resulting in much more effective in the protection of DNA damage induced by lipid peroxidation (Ramadas et al, 2015). It also possesses hepatoprotective action in respect of certain toxic substances such as carbon tetrachloride, preventing the consequent increase in bilirubin, transaminases and alkaline phosphatase, indices of liver damage. During the last decades, several studies have explored the beneficial effects of curcumin as an anti-inflammatory agent, already known in Ayurvedic medicine, which can decrease inflammation and also play a role in cancer treatment (Shanmugam et al, 2015; Takeyama et al, 2015). It is known that peroxisome proliferator-activated receptors (PPARs) are transcription factors that play an important role in the regulation of genes involved in lipid utilization and storage, lipoprotein metabolism, adipocyte differentiation, and insulin action (Riaz et al, 2000). A review by Jacob et al. (2007) highlights the importance of curcumin as an anti-inflammatory agent and suggests that the beneficial effect of curcumin is mediated by the upregulation of peroxisome proliferator-activated receptor-γ (PPAR-γ) activation. Moreover, there is an increasing interest in curcumin as a cardiovascular disease (CVD) protective agent via decreased blood total cholesterol and low-density lipoprotein-cholesterol (LDL-cholesterol) level. A study by Kim and Kim (2010) has investigated the potential mechanism in the hypocholesterolemic effect of curcumin by measuring cholesterol 7a-hydroxylase (CYP7A1), a rate limiting enzyme in the biosynthesis of bile acid from cholesterol, at the mRNA level. The study results suggest that curcumin reduces concentration of blood cholesterol via induction of the CYP7A1 gene expression. Furthermore curcumin is capable of inhibiting *in vitro* COX1 and COX2 enzymes involved in inflammatory reactions and in osteoarthritis pathologies. Inflammation plays an important role in the development of most diseases: cardiovascular disease, cancer, lung disease, neurological, autoimmune, various forms of arthritis, diabetes. According to the Indian professor Bharat Aggarwal NF-k□ (nuclear transcription kappa beta factor) plays an important role in most of the disease and its inhibition may suppress inflammation (Hoesel et al, 2012); according to a hypothesis, the majority of tumors, the active NF-k□ factor, which in turn promotes the proliferation and metastasis of tumors. The incidence of four very common cancers, breast colon, prostate and lung, the lung is up to ten times lower in India, where significant quantities of turmeric are consumed daily, as compared for example to the United States (Anthwal et al, 2014).

In the literature, many mechanisms of action for the antitumor activity of curcumin have been described; in particular, this activity goes through several mechanisms, such as inhibition of tumor cell proliferation, induction of apoptosis, inhibition of cell transformation from normal to tumor, inhibition of the formation of blood vessels that feed the tumor (anti-

angiogenic effect), inhibition of invasiveness and metastasis suppression and inflammation (Perrone et al, 2015).

Curcumin has antibacterial activity and is used not only to prevent deterioration of foods, but also for the treatment of small wounds to prevent infection and facilitate tissue reconstruction and especially for the skin protection against damage caused by anticancer radiotherapy.

Depression, dementia, neurodegenerative diseases such as Alzheimer's disease, are on the rise because of the aging population in developed countries represents a serious problem for health care purposes.

A study on rats who had behavioral problems, such as poor mobility, showed that co-administration of curcumin and piperine has achieved significant effects both physically and increasing the mobility of the animals with decreased activity of monoamine oxidase and increase of serotonin and dopamine in the brain. The presence of piperine increased bioavailability of curcumin enhancing the effects, and this experience has provided the conditions for the use of curcumin and piperine in the treatment of depressive disorders (Dhutani et al, 2009).

Recent studies on curcumin have shown the anti-amyloidogenic activity, anti-inflammatory, antioxidant and metal chelator that may have neuro-protective effects. In particular, the hydrophobicity of the molecule might allow the passage through the blood brain barrier and subsequent accumulation in the brain, but the brain concentration of curcumin was insufficient due to low bioavailability, mainly due to its shallow water solubility, poor stability in solution, and rapid intestinal deletion even though many efforts have been made to improve its bioavailability. Increasing doses of administration, practical applied in many *in vivo* studies and clinical trials, do not seem an optimal solution, although curcumin possesses low toxicity, because research have always been conducted for limited and not long-term periods (Chin et al, 2013). The only certainty is determined by the fact that curcumin is not toxic to humans of up to 8 g/day dose and prevents the formation of amyloid plaques responsible for the disease (Hsu et al, 2007). The American Food and Drug Administration classifies the curcumin as a GRAS substance (Generally Recognized As Safe), because not teratogenic and free of side effects, at least within the allowable dosages. Only in special cases, such as pregnant women, people with bleeding disorders and patients with gallstones the restriction is necessary.

Conclusion:

Currently there are no toxicological studies on the long-term curcumin administration. However, some research has demonstrated the absence of toxicity in humans with dosing this active principle for short periods of time. Therefore, for its beneficial and healing properties, curcumin

obtained by the described extraction method, may be used as a natural dietary supplement. From the results obtained, it can be stated that the quality of the extracted compounds is quite similar to that of commercial standards and the method is economically advantageous. The extraction process by means of Naviglio extractor is carried out at room temperature, using a pressure increase in the extracting liquid on the solid matrix to be extracted, with a lower risk of degradation of the extracts, as it is in other conventional systems.

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