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A study on Turmeric (*Curcuma longa* L.): Multifunctional agents for the management of oxidative damage, neurodegeneration and cancer

Sevgi Gezici

ABSTRACT

The rhizome of *Curcuma longa* L, (turmeric, curcumin) has been widely used in therapeutic purposes for acne, heal wound, prevent skin damage, reduce cholesterol, treat diabetes, and control blood pressure. From this point of view, this research was aimed to investigate its biological properties including antioxidant, anticancer and neuroprotective properties of the turmeric rhizomes. The rhizomes of turmeric were extracted with methanol-MeOH and distilled water-dH_2O, and subjected to various assays. Neuroprotective potentials of the extracts were tested through enzyme inhibitory assays on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), which are closely linked to pathogenesis of Alzheimer's disease. Their anticancer activities were evaluated using MTT assay against A549, MCF-7, HeLa human cancer cells, and non-tumorous HUVECs. *In vitro* methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) were performed to reveal antioxidant capacities of the extracts. Total polyphenolic compositions of the extracts were also identified spectrophotometrically. The turmeric extracts were found to have rich polyphenolic quantities, particularly the MeOH-extract possessed higher total polyphenolic contents than the dH_2O-extract. The extracts demonstrated the remarkable inhibition on both of the cholinesterase enzymes even at the lowest concentration (100µg mL^{-1}). Moreover, they showed higher enzyme inhibition against AChE, comparing with that of BChE enzyme. In general, a significant correlation was observed between the total antioxidant capacities and neuroprotective potentials of the extracts from turmeric rhizomes. As for the anticancer activity, the extracts were found as a natural anticancer agent with the IC_{50} values ranged from 13.01±0.16 to 26.72±1.04 µg mL^{-1}. In the light of the findings of the presented research, it is clearly concluded that turmeric is an important natural source to fight oxidative stress related diseases, with its excellent cholinesterase-inhibiting properties; strong antioxidant capacities as well as remarkable anticancer activities.

Key words: *Curcuma longa* L.; turmeric; Alzheimer's disease; oxidative stress; neuroprotective; anticancer

1. INTRODUCTION

Turmeric (*Curcuma longa* Linn.), belonging to Zingiberaceae (ginger) family, is a widely used spice, food preservative and coloring material. The rhizome of the plant, known as 'Golden Spice' because of its brilliant yellow color, is a very popular spice in Africa, Thailand, Pakistan, India, and Turkey.[1-3]

C. longa L. has a long history of use in traditional medicine for treatment of various disease and disorders including cough, diabetic ulcers, hepatic diseases, biliary disorders, rheumatism, sinusitis and anorexia, owing to its wide range of biological properties such as antioxidant, antimicrobial, anti-inflammatory, anticoagulant, antidiabetic and immunomodulatory.[1,4] It is rich in terms of bioactive secondary metabolites that includes curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Among them, curcumin, the most important constituent of *C. longa* L., is responsible for its yellow color.[5,6]

The formation of Reactive Oxygen Species (ROS), including superoxide ion (O_2), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2), have often been reported to induce DNA damage, protein carboxylation, and lipid peroxidation, causing a variety of chronic health disturbances and diseases, such as cancer, ageing, cardiovascular diseases, Alzheimer's and Parkinson's diseases. Recent researches have indicated that several herbal plants can offer alternative sources of dietary ingredients to promote human health and might open promising opportunities for the treatment of troublesome diseases and infections.[7,8] Although, there are so many methods for combatting neurodegenerative diseases and cancer cases, they cannot always provide effective treatments and mediations. In this context, an extensive research on developing new treatment strategies for management oxidative damage are still needed nowadays. Since ancient times, natural products (NPs), widely originated from medicinal plants and their bioactive compounds, have been used for cure and treatment of many ailments and diseases in Anatolian folk medicine.[9-11]

Various medicinal and aromatic plants (MAPs) and their isolated bioactive compounds were analyzed to reveal their anticancer, antiproliferative, anticholinesterase, antityrosinase, antioxidant, etc. capacities by our research group.[12-25] The main purpose of these researches are to contribute to discover new and effective natural products for prevention and treatment of oxidative stress-related diseases and disorders. Take into consideration our previous researches on MAPs and plant-derived natural products, total polyphenolic contents, *in vitro* antioxidant effects, neuroprotective and anticancer activities of the extracts obtained from the rhizomes of *C. longa* L. were aimed to evaluate in the presented research.

2. MATERIAL AND METHODS

2.1 Plant Material

The dried rhizomes of *C. longa* L. were obtained from a local herbal market. Taxonomic identification of the plant was performed according to the Flora of Turkey, and a voucher specimen was deposited in the Biology Department of Kilis 7 Aralik University, Kilis-Turkey (herbarium number: 0108). Plant material and its powdered rhizomes were given in the Figure 1.

2.2 Crude Extract Preparation

To prepare crude extracts, air dried samples (50 g) of the rhizomes of *C. longa* L. were extracted with methanol (MeOH), and distilled water (dH_2O) by using maceration method for 16-24 hours at the room temperature. The extraction procedures were performed as described in our previous research.[13] Extraction yields of the methanol and water extracts of the rhizomes from turmeric were determined as 12.36% and 8.02% (w/w), respectively. The methods of used herein were summarized as graphical abstract in the Figure 2.

A **B**

Fig. 1: Rhizomes (A) and rhizome powder (B) of turmeric (*C. longa* L.)

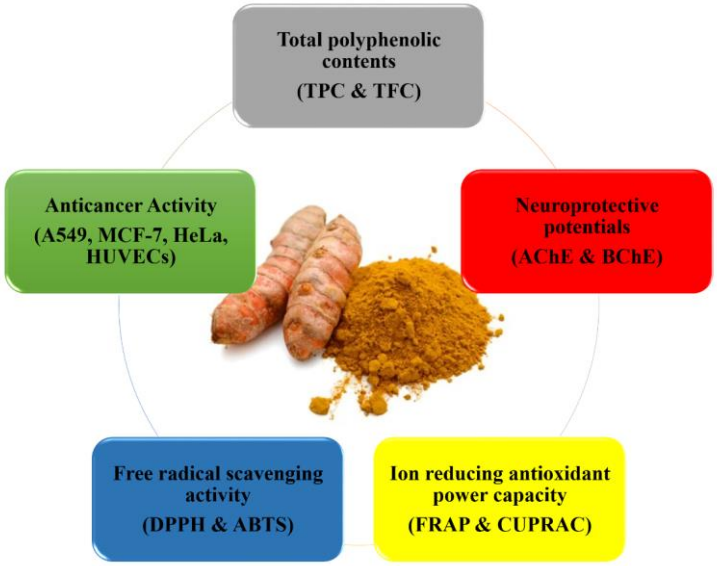

Fig. 2: Graphical abstract of the presented research

2.3 Determination of Total Polyphenolic Contents

Phenolic compounds in total were determined in accordance with slightly modified Folin-Ciocalteau's method.[13,26] Absorption was measured at 760 nm at a using a 96-well microplate reader (VersaMax Molecular Devices, USA). Total flavonoid content of the extracts was calculated by aluminum chloride colorimetric method.[13,27] A number of dilutions of quercetin were obtained to prepare a calibration curve. Absorbance of the reaction mixtures was measured at wavelength of 415 nm with a using a 96-well microplate reader (VersaMax Molecular Devices, USA). The total phenol and flavonoid contents of the extracts were expressed as gallic acid and quercetin equivalents (mg g^{-1} extract), respectively.

2.4 Antioxidant Activity Assays

In vitro antioxidant methods including 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and cupric ion reducing capacity (CUPRAC) were performed to reveal antioxidant activity of the extracts.[13,18,21,22] The extracts and commercial antioxidant standards were dissolved in DMSO at different concentrations, ranging from 100 µg mL^{-1} to 1000 µg mL^{-1} for the antioxidant assays.

2.5 Neuroprotective Activity Assays

Neuroprotective potentials of the extracts against AChE (acetylcholinesterase) and BChE (butyrylcholinesterase) were evaluated in that study. For the enzyme inhibition assays, the extracts were dissolved in DMSO at different concentrations, ranging from 100 µg mL^{-1} to 400 µg mL^{-1}. AChE and BChE inhibitory activity of the samples was measured by slightly modified spectrophotometric method of Ellman et al. (1961).[28] Electric eel AChE (EC 3.1.1.Sigma, St. Louis, MO, USA) and horse serum BChE (EC 3.1.1. Sigma, St. Louis, 7 MO, USA) were used, while acetylthiocholine iodide and butyrylthiocholine chloride (Sigma, St. Louis, MO, USA) were employed as substrates of the reaction. 5,5´-Dithio- bis(2-nitrobenzoic)acid (DTNB, Sigma, St. Louis, MO, USA) was used for the measurement of the anticholinesterase activity. All the conditions and calculations were same as described in our previous publications.[13,17] The data was obtained from the assays, expressed as mean and standard deviation of mean (mean±SD). The percentage of enzyme inhibition on AChE and BChE was calculated as $[(Abs_{control} - Abs_{sample})/Abs_{control} \times 100]$, where $Abs_{control}$ value is the absorbance of the control solvent (blank), and Abs_{sample} is the absorbance of the tested sample (plant extract or positive control in the solvent) in the presence of enzyme. Galanthamine hydrobromide (Sigma, St. Louis, MO, USA) was used as the reference drug.

2.6 Human Cancer Cells and Anticancer Activity Assay

A549 (lung carcinoma), MCF-7 (breast adenocarcinoma), HeLa (cervical cancer) human cancer cells and non-tumorous HUVEC (human umbilical vein endothelial cell) cells, obtained from the American Type Culture Collection (ATCC, USA) were used to evaluate the potential anticancer and cytotoxic activities of the rhizomes extracts from turmeric. The A549 and HeLa cancer cells were cultured on Roswell Park Memorial Institute Medium (RPMI, ThermoFisher Scientific), and the other cells were grown in Dulbecco's modified Eagle medium (DMEM): Ham's F12 nutrient medium (1:1) (ThermoFisher Scientific) in the flasks at 37°C in a humidified CO_2 (5%) incubator. The cell growing conditions and supplements were used as same described in our previous publications (Gezici et al., 2017; Gezici, 2018; Gezici, 2019). In order to determine anticancer activities of the turmeric extracts, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed as described by Mosmann (1983)[29] with minor modifications.[12,16] The absorbance was measured at 570 nm with a Thermo Lab systems 408 Multiskan multiplate spectrophotometer, and the dose response curve was used to generate the IC_{50} (µg mL^{-1}) values for each cells.

2.7 Statistical analysis

The experiments were done in quadruplicate, and the results were expressed as mean and standard deviation values (mean ± SD). Statistical differences between the references and the sample groups were evaluated by ANOVA (one way). Correlations were performed using the correlation and regression in the EXEL program. P value of <0.05 was considered to be statistically significant, $p < 0.01$ and $p < 0.001$ were considered to be very significant.

3. RESULTS AND DISCUSSION

3.1 Results of Total Polyphenolic Contents

Phenolic compounds such as phenolic acids and flavonoids are reported to be involved in various biochemical activities like antioxidant, antimicrobial, antithrombotic, antiartherogenic, antiinflammatory, anticarcinogenic and antimutagenic. In the current research, total phenolic (TPC) and flavonoid compositions (TFC) of the extracts were determined spectrophotometrically. Gallic acid (GA) and quercetin (Q) equivalent used as commercial standards.

In case of total polyphenolic contents, extracts from turmeric rhizomes possessed higher total phenolic quantities than flavonoid quantities in total. As seen in the Table 1, both water and methanol extracts of turmeric had rich polyphenolic contents, among the extracts, the methanol extract was found to possess of higher total phenolic and flavonoid contents (1764±0.16 mg GAE/100 g and 1342±0.25 mg QE/100g, respectively) comparing with that of the water extract.

3.2 Results of Antioxidant Capacity

Antioxidant activity of the extracts obtained from the powdered rhizomes of turmeric were screened by using radical scavenging against DPPH and ABTS radicals, and ion reducing antioxidant power on FRAP and CUPRAC. The extracts exhibited significant free radical scavenging activities on DPPH, and ABTS, and ion reducing power capacity for FRAP and CUPRAC.

MeOH extract, which showed a higher TPC and TFC than that of the dH_2O extract, was found the most effective in all the

antioxidant assays. Scavenging activity of the MeOH extract on DPPH was determined as 78.81±0.04% inhibition when it was found as 71.68±0.11% inhibition for the dH_2O extract. On the other hand, the MeOH extract was found to have 83.12±0.36% inhibition on ABTS, which was found higher than the dH_2O extract. As presented in the Figure 3, inhibition percentage of the extracts on DPPH and ABTS were compared with ascorbic acid, as a standard antioxidant. As regards of FRAP and CUPRAC ion reducing antioxidant power capacities of the rhizomes of *C. longa* L., it is revealed that the tested extracts were showed the weakest antioxidant activity on FRAP with the absorbance values of 1.862±0.006, and 1.408±0.021 for the methanol and water extracts, respectively. The obtained absorbance values from the turmeric extracts were given as comparing chlorogenic acid and trolox, standard antioxidants (Table 2).

3.3 Results of Neuroprotective Potentials

Neuroprotective activity of the extracts was assessed through enzyme inhibition assays on cholinesterase enzymes. The rhizomes extracts of the plant subjected to enzyme inhibitory assays on cholinesterase enzymes, which are closely associated with pathogenesis of neurodegenerative disease.

As given in Table 3, the turmeric extracts exerted the remarkable inhibition both on AChE and BChE enzymes. Furthermore, the higher cholinesterase inhibitory activity was observed against AChE, compared to BChE at the tested concentrations. As consistent with antioxidant activities, the methanol extract was exhibited higher enzyme inhibitory capacities than compared to water extract. Meanwhile, the water and methanol extracts of turmeric were showed 78.14±0.13% to 84.01±0.10% inhibition on AChE, and 44.52±0.48% to 58.72±0.26% inhibition on BChE, respectively (Table 3).

3.4 Results of Anticancer Activity

MTT assay was conducted to evaluate the cytotoxic activities of the rhizomes extracts from *C. longa* L. on three human cancer cell lines (A549, MCF-7, and HeLa), compared to cytotoxic activity of non-tumorous HUVECs. Anticancer activity results are given as Figure 4, regarding of IC_{50} values after 48h treatment period (Fig 4).

The results demonstrated that the turmeric extracts showed significant cytotoxicity against the tested cancer cells, even at lower dose and minimum exposure time. Among the tested cancer cells, the highest cytotoxic effect was found towards HeLa cells, whilst the lowest one was determined against A549 cells. The MeOH and dH_2O extracts obtained from the rhizomes of turmeric were exerted the highest anticancer activity on HeLa cells, with the IC_{50} values 13.01±0.16 and 15.28±0.30 μg mL^{-1} (at 800 μg mL^{-1} concentration), respectively. On the other hand, the lowest anticancer activity was observed towards A549 cells in the MeOH extract of turmeric (IC_{50} = 26.72±1.04), which was followed by dH_2O extract of turmeric on MCF-7 cells (Fig 4). The findings on cytotoxicity could clearly revealed that the rhizome extracts of *C. longa* L. possess remarkable anticancer activity against the tested cancer cells in a dose dependent manner, therefore turmeric have significant potential in the prevention of cancer development.

Table 1. Total polyphenolic contents of the extracts at 1000 μg mL^{-1}

Extract type	**Total phenolic content (TPC) (mean ± SD)**	**Total flavonoid content (TFC) (mean ± SD)**
Methanol	1764 ± 0.16***	1281 ± 0.60***
Water	1342 ± 0.25***	1029 ± 1.08**

SD: Standard deviation (n=3).
***p value of < 0.01; ***p value of < 0.001.*

Table 2. Reducing power capacity of the extracts for FRAP and CUPRAC

Extract type	**FRAP**	**CUPRAC**
Methanol	1.862 ± 0.006**	3.640 ± 0.028***
Water	1.408 ± 0.021***	3.015 ± 0.012*
Chlorogenic acid[a]	3.628 ± 0.002	---
Trolox[b]	---	2.148± 0.008

The values were given as absorbance values, and higher absorbance indicates greater antioxidant activity.
[a]Chlorogenic acid was used as a standard antioxidant for FRAP assay.
[b]Trolox was used as a standard antioxidant for CUPRAC assay.
**p value of < 0.05; **p value of < 0.01; ***p value of < 0.001.*

Table 3. Neuroprotective effects against AChE and BChE at 800 µg mL^{-1}

Extract type	Acetylcolinesterase (AChE) (Inhibition % ± SD)	Butyrylcolinesterase (BChE) (Inhibition % ± SD)
Methanol	**84.01 ± 0.10****	**58.72 ± 0.26***
Water	**78.14 ± 0.13*****	**44.52 ± 0.48****
Galantamine[a]	**88.60 ± 0.02**	**84.12 ± 0.09**

[a] ***Galanthamine; commercial standard for AChE and BChE inhibition.***

****p value of < 0.05; **p value of < 0.01; ***p value of < 0.001***

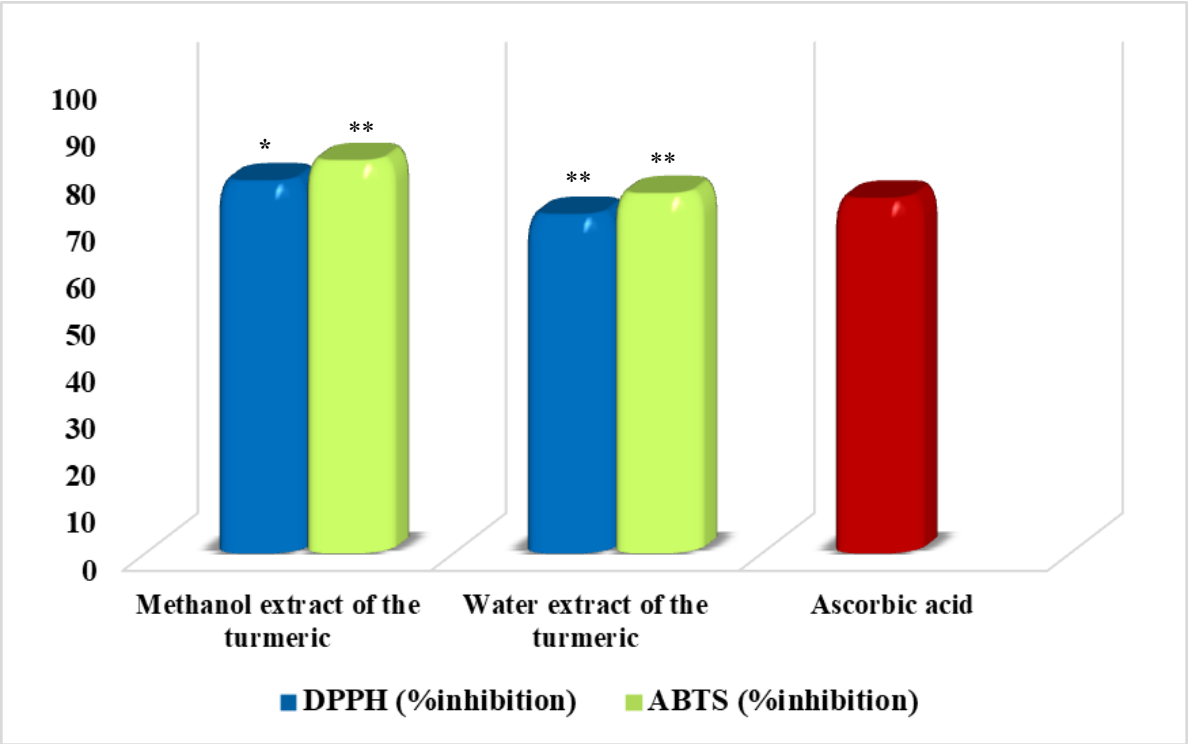

Fig.3: Free radical scavenging activity of the extracts against DPPH and ABTS radicals

The values were presented as percentage of inhibition obtained from three independent experiments (n=3). Ascorbic acid was used as a standard antioxidant for DPPH and ABTS assays.
****p value of < 0.05; **p value of < 0.01.***

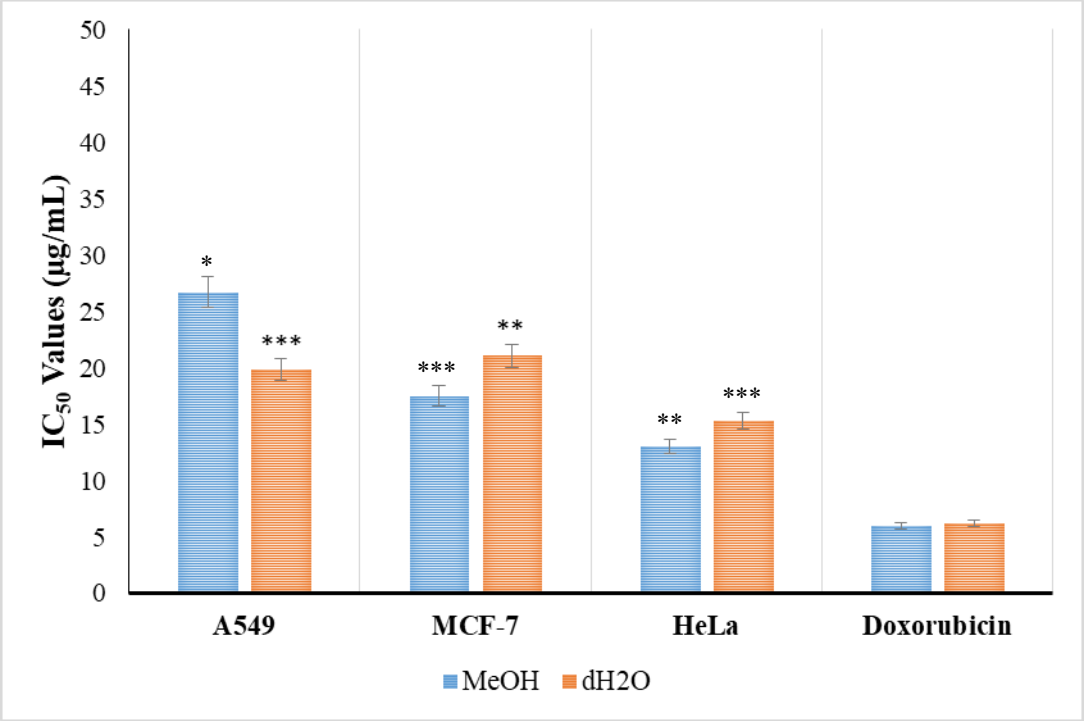

Fig. 4: Anticancer activity of turmeric extracts against A549, MCF-7, and HeLa human cancer cells for 48h

The data was expressed as IC_{50} values ± SD (n=3).
Doxorubicin, commercial anticancer agent as a positive control.
**p value of < 0.05; **p value of < 0.01; ***p value of < 0.001.*

4. CONCLUSION

As far as our literature survey, there has been no study so far examining neuroprotective and anticancer potentials of the rhizomes extracts obtained from *C. longa* L. (turmeric) combining and correlated with the antioxidant assays and polyphenolic quantities. From the point of view, the results presented in this study could be the first report for the literature. The results revealed that the rhizomes of turmeric have remarkable cholinesterase-inhibiting properties and anticancer activities, as well as excellent free radical scavenging and ion reducing power antioxidant capacities. Consequently, the extracts from turmeric appear to be a essential natural source having promising inhibitory bioactive secondary metabolites, are worth to perform further *in vivo* and clinical investigations that are under further investigations in our laboratory.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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