

In vitro antioxidant and anticholinesterase activities of ethanolic turmeric crude extract

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Abstract

This study was aimed at exploring the antioxidant and anticholinesterase effect of ethanolic turmeric crude extract. The antioxidant activity was determined by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging activity, (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) (ABTS) and ferric reducing antioxidant power (FRAP) values. Ethanolic crude extract of turmeric showed good antioxidant and anticholinesterase activities (% I); the activity was, 80.2±1.30%, 75.6±1.23% and 10.43±0.28% for ABTS, DPPH and FRAP, respectively, and 69.0±1.85% and 70.5±1.11% for AChE and BChE, respectively. However, the maximum% I for ABTS, DPPH and FRAP, that is, 87.2±1.34%, 86.0±1.59% and 19.36±0.37 µg FSE, respectively, were shown by positive control ascorbic acid, and for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition, 86.69±1.24 and 89.3±1.01%, respectively, by galanthamine. Turmeric provides a potential natural source of bioactive compounds and is valuable to human health.

1. Introduction

Oxidation is the most important contributing factor for many diseases and disorders in human beings. The antioxidant inhibits oxidative damage by the reaction of an electron with a free radical that modify the cellular lipids, deoxyribonucleic acid (DNA), protein, and polysaccharides. It is necessary to maintain a balance between antioxidant concentration and free radicals for proper physiological functions. (Lobo *et al.*, 2010; Nimse and Pal, 2015; Nor *et al.*, 2018). Oxidative stress is the imbalance between oxidants and antioxidants in a biological system that occurs due to improper functioning of the antioxidant system or excessive production of reactive oxygen species (ROS). Oxidative stress leading to the free radical attack on neuronal cells play a dreadful role in the development of neurodegeneration. It has been associated with the development of neurodegenerative diseases like Alzheimer's disease (AD), Parkinson's disease (PD) (Liu *et al.*, 2017). Among all the neurological diseases, AD is the indefinite neurodegenerative disease and is the greatest common cause of dementia. AD is linked with synapses loss, synaptic dysfunction of the synaptic cleft, abnormal function of mitochondria and inflammatory

stress that may cause the multi neurotransmitters deficiency like acetylcholine, serotonin, noradrenalin and somatostatin. Acetylcholine is involved in the transmission of signals in the synapse and its pharmacological act is terminated initially by AChE and then by BChE. So, the inhibitors of these metabolizing enzymes are important substitutes in the treatment of AD (Kamal *et al.*, 2015; Hv and Raj, 2020).

It is reported earlier that the chemical components of medicinal plants have shown antioxidant activity by inhibiting free radicals and the process of oxidation. These plant antioxidants break the chain reactions of free radicals. A variety of chemical compounds like polyphenols, ascorbic acid etc. are responsible for antioxidant activity. They inhibit the lipid peroxidation by inactivation of lipoxygenase, to scavenge active oxygen species and free radicals by proliferating a reaction cycle and heavy metal ions chelation (Kumar *et al.*, 2013; Dinnimath and Jalalpure, 2018). The natural products obtained from the plants have strong potential against neurodegenerative disorders (Nour *et al.*, 2014). Various studies have shown that turmeric ameliorates the symptoms related to various diseases (Amalraj *et al.*, 2017). Turmeric is one of the most common medicinal

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herbs, with a broad range of pharmacological properties such as antioxidant, anti-inflammatory (Gupta *et al.*, 2013; Krup *et al.*, 2015; Hv and Raj, 2020). Antiangiogenic (Maheshwari *et al.*, 2006), antiprotozoal, antivenom, antimalarial, antimicrobial, antirheumatic, antiproliferative, anti-cancerous and antiaging properties. It has also treated ulcers, various skin diseases, parasitic infections, anti-immune diseases and also cured the colds and flu symptoms (He *et al.*, 2015; Tanvir *et al.*, 2017).

The DPPH radical dissolves only in the organic medium in contrast to ABTS cationic radical is soluble in both aqueous and organic media. The ABTS assay can, thus, be used to screen both hydrophilic and lipophilic samples (Bibi Sadeer *et al.*, 2020).

This study is focused on the antioxidant and anticholinesterase potential of ethanolic crude extract of Turmeric. Antioxidant activities of Turmeric crude extract were carried out by DPPH free-radical scavenging, ABTS and FRAP assays. The drug potentials of turmeric crude extract for Neurodegenerative diseases were controlled with their inhibitory effect against AChE and BChE enzymes.

2. Materials and methods

2.1 Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH, 95% purity), 5,5'-dithiobis [2-nitrobenzoic acid] (DTNB), 2,2'-azino-di- (3-ethylbenzthiazoline sulfonic acid) diammonium salt (ABTS, ≈98 purity), 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid (HCl), potassium persulfate ($K_2S_2O_8$), ferrous chloride ($FeCl_3$), sodium trihydrate (H_6NaO_3), acetic acid (CH_3COOH), ascorbic acid ($C_6H_8O_6$), acetylcholinesterase (AChE), acetylthiocholine iodide (ACTI) and galanthamine were purchased from Sigma-Aldrich. Absolute ethanol (≥99.4%) and methanol (99%) were purchased from a local supplier. Turmeric rhizomes were purchased from local vendors.

2.2 Preparation of turmeric crude extract

Turmeric was washed with distilled water, peeled and cut into small pieces. The pieces were put into the oven for 24 hrs at 37°C for drying and then make a powder. The protocol described by Tanvir *et al.* (2017) was used with modification for the Turmeric crude extraction from *Curcuma longa*. In brief, 20 g of Turmeric powder was extracted with 300 mL of absolute ethanol in a flask. The extract was filtered using Whatman filter paper No. 1. The filtered extract was concentrated to dryness in a hot air oven at 40°C for 48 hrs to obtain a dark orange solid mass. The percentage yield was calculated by equation 1. A 10 mg/mL stock

solution of the dried Turmeric crude extract was prepared in absolute ethanol.

$$\text{Yield (\%)} = \frac{\text{Weight of Tur - CE (g)}}{\text{Weight of Turmeric Used (g)}} \times 100 \quad (1)$$

2.3 Evaluation of antioxidant activity using in vitro assay

Antioxidant activity was evaluated by DPPH, ABTS and FRAP assay. All analyses were performed in triplicate.

2.3.1 DPPH free radical scavenging activity

DPPH free radical scavenging activity was determined by following the protocol adopted from Marinova and Batchvarov, (2011) with modifications. In brief, 500 μL of 0.1 mM DPPH (dissolved in ethanol) was mixed separately with different concentrations (100 μL, 200 μL, 300 μL, 400 μL and 500 μL) of Turmeric crude extract diluted to a total volume of 1 mL with distilled water where needed. The mixture was vigorously shaken and kept at room temperature for 30 mins in a dark place. The different concentrations of freshly prepared ascorbic acid were used as the standard antioxidant compound. The absorbance was recorded at 517 nm, absolute ethanol was used as a reference standard. The mixture of 1 mL ethanol and 1 mL DPPH solution was used as a control. Radical scavenging activity was expressed as percent inhibition.

$$\% I = \frac{C - S}{C} \times 100 \quad (2)$$

Where I is the percentage inhibition, C is the absorbance of control and S is the absorbance of the sample.

2.3.2 ABTS free radical scavenging activity

ABTS free radical scavenging assay was performed by following the procedure described by (Re *et al.*, 1999) after modification. ABTS reagent was prepared by mixing H_2O and ABTS to get a 7 mM concentration with 2.45 mM potassium persulfate ($K_2S_2O_8$) and stored in the dark at room temperature for 12-16 hrs. The ABTS solution was diluted in ethanol to get an absorbance of 0.750 ± 0.025 at 734 nm. Then, 2 mL of ABTS solution was added to the test solutions at different concentrations (100 μL, 200 μL, 300 μL, 400 μL and 500 μL, diluted to a total volume of 1 mL with distilled water). The reaction mixture was vortexed for 10 s and allowed to stand at room temperature in the dark. The absorbance was measured after 6 mins and calculated the percentage of radical scavenging with reference to blank containing no antioxidant. The different concentrations of freshly prepared ascorbic acid were used as the standard antioxidant compound. ABTS•1 free radical scavenging activity was expressed as percent inhibition (% I)

calculated as described in equation 1.

2.3.3 FRAP ferric reducing antioxidant power

The FRAP assay was done to follow the method of Benzie and Strain (Benzie and Strain, 1996) with modifications. The stock solutions have 300 mM acetate buffer (310 mg sodium trihydrate, 1.6 mL of acetic acid, pH 3.6 and volume was 100 mL), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCl, and FeCl₃.6H₂O was 20 mM. The solution was prepared fresh by mixing 30 mL acetate buffer, 3 mL TPTZ solution, and 3 mL FeCl₃.6H₂O solution. Different concentration (100 µL, 200 µL, 300 µL, 400 µL and 500 µL diluted to a total volume of 500 µL with distilled water, respectively) of turmeric crude extract were allowed to react with 2 mL of the FRAP solution for 30 min in the dark condition. The coloured solution reading was taken by UV-vis at 593 nm. The different concentrations of freshly prepared ascorbic acid were used as the standard antioxidant compound. Results were expressed in microgram ferrous sulphate equivalent (µg FSE).

2.4 Evaluation of anticholinesterase activity using in vitro assay

The AChE and BChE activity assay was carried out as described by Perry *et al.* (2000) with modifications. In brief, different concentrations of Turmeric crude extract were added to Eppendorf tubes. After that, 595 µL Tris-HCl buffer pH 8.0 and 20 µL 0.1 U/mL AChE or 0.9 U/mL BChE were added into the tubes followed by incubation at room temperature for 15 mins. Then, 125 µL DTNB 3 mM was added followed by the addition of 10 µL ATCI 14 mM to initiate the reaction. The hydrolysis of ATCI by AChE or BChE produces thiocholine which readily reacts with DTNB and a yellow product, 5-thio-2-nitrobenzoic acid anion, was released whose production was recorded spectrophotometrically at 412 nm for 10 mins at room temperature. A blank, containing the reagents but no enzyme, was used as a control. Different concentrations of galanthamine, that is, 2, 4, 6, 8 and 10 µg dissolved in 100 to 500 µL were used as a positive control. The method described by Ellman *et al.* (1961) was used to calculate the rate of AChE and BChE hydrolysis. The % inhibition was calculated by the following formula:

$$\% I = \frac{E-S}{E} \times 100 \quad (3)$$

Where % I is the percent inhibition, E is the activity of enzyme without test compound and S is the activity of the enzyme with the test compound.

3. Results and discussion

Turmeric powder was 20 g that was used to prepare Turmeric crude extract. After extraction, the % yield of turmeric crude extract was recorded at 17.5%.

3.1 Evaluation of in vitro antioxidant activity

The reducing property is associated with the presence of reactants. The antioxidant activity of reactants is takes place by donating the hydrogen atom and breaking the free radical chain. The reducing ability of the extracts are due to the presence of polyphenols. These polyphenols donating the electrons and reacting with free radicals to change them to stable products and terminate radical chain reaction (Loganayaki *et al.*, 2013).

DPPH is a stable and typical method used for antioxidant potential determination in a short time period. The scavenging ability was evaluated with various concentrations of ethanolic Turmeric crude extract. The ethanolic turmeric crude extract shows good antioxidant activity. The dose-response bar chart of DPPH and ABTS is shown in Figure 1 and Figure 2 respectively, which indicate that by increasing the concentration of extract the antioxidant potential increases.

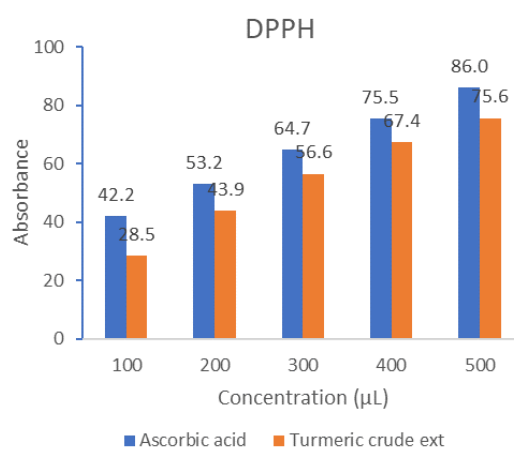


Figure 1. DPPH of Turmeric crude extract and ascorbic acid at different concentrations.

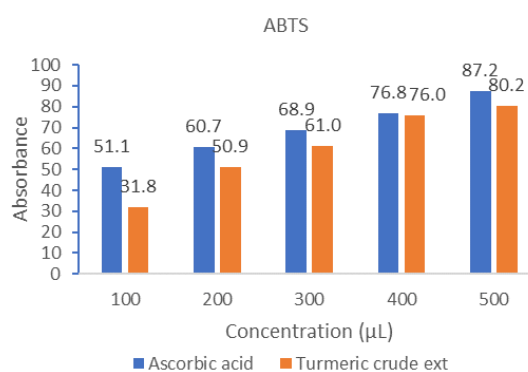


Figure 2. ABTS of Turmeric crude extract and ascorbic acid at different concentrations.

The reducing power of the extract increased with an increase in the concentration of the extract. The FRAP assay is based on the capability of antioxidants to reduce Fe^{3+} to Fe^{2+} with the help of TPTZ to form an intense blue Fe^{2+} -TPTZ complex at 593 nm absorption maxima. Figure 3 illustrates the total antioxidative power of the crude extracts of Turmeric in comparison with ascorbic acid (positive control), FeSO_4 (standard for calibration). The FRAP value is expressed as $\mu\text{g FSE}$. The results show that the extract exhibits significant ferric reducing capacity at 500 μL extract concentration the FRAP value is $10.43 \pm 0.28 \mu\text{g FSE}$. Table 1 shows the value of DPPH, ABTS, FRAP of Turmeric crude extract at different concentrations and their control Ascorbic acid.

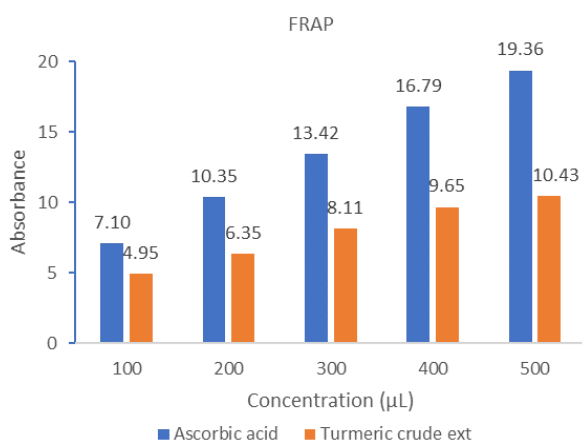


Figure 3. FRAP of Turmeric crude extract and ascorbic acid at different concentrations.

3.2 Evaluation of *in vitro* anticholinesterase activity

Naturally, occurring cholinesterase are of particular interest and currently considered as the notable alternative therapeutic agent for the treatment of neurodegenerative disorders like AD (Tariot and Federoff, 2003; Natarajan *et al.*, 2013). The Turmeric crude extract was tested for their anti-AChE and anti-BChE activities at different concentrations (100 μL , 200

μL , 300 μL , 400 μL , 500 μL) by a modified Elman's method (Owokotomo *et al.*, 2015), using galanthamine as the standard. Table 2 show the % inhibitory activity of the extract against AChE and BChE. The dose-response bar chart of AChE and BChE inhibitory activities of Turmeric crude extract under different temperature conditions have been shown in Figure 4 and Figure 5, respectively.

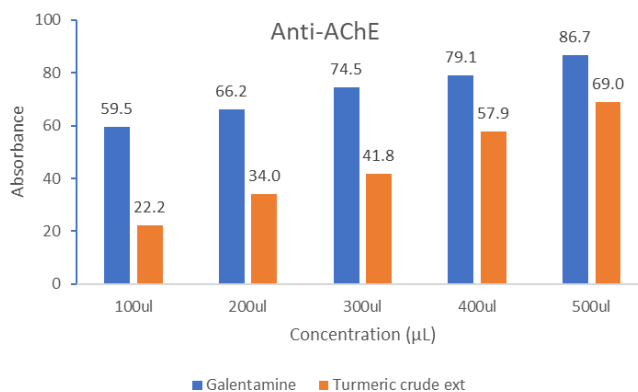


Figure 4. Anti-AChE of Turmeric crude extract and galantamine at different concentrations.

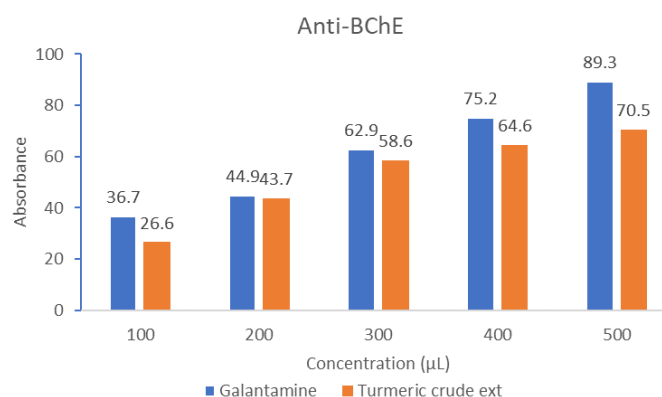


Figure 5. Anti-BChE of Turmeric crude extract and galantamine at different concentrations.

Table 1. Values of DPPH, ABTS and FRAP and their controls at different concentrations of Turmeric crude extract.

Assay	Concentration (μL)				
	100	200	300	400	500
DPPH (%I)	28.5 \pm 1.45	43.9 \pm 1.27	56.6 \pm 1.12	67.4 \pm 1.74	75.6 \pm 1.23
Control	42.2 \pm 1.16	53.2 \pm 1.50	64.7 \pm 1.06	75.5 \pm 1.22	86.0 \pm 1.59
ABTS (%I)	31.8 \pm 0.79	50.9 \pm 1.03	61.0 \pm 0.98	76.0 \pm 1.03	80.2 \pm 1.30
Control	51.1 \pm 2.17	60.7 \pm 1.97	68.9 \pm 1.84	76.8 \pm 1.51	87.2 \pm 1.34
FRAP ($\mu\text{g FSE}$)	4.95 \pm 0.26	6.35 \pm 0.38	8.11 \pm 0.28	9.65 \pm 0.33	10.43 \pm 0.28
Control	7.10 \pm 0.36	10.35 \pm 0.37	13.42 \pm 0.45	16.79 \pm 0.30	19.36 \pm 0.30

Table 2. Values of anti-AChE, anti-BChE and their controls at different concentrations of Turmeric crude extract.

Assay	Concentration (μL)				
	100	200	300	400	500
Anti-AChE activity (% I)	22.2 \pm 0.78	34.0 \pm 1.50	41.8 \pm 2.31	57.9 \pm 1.21	69.0 \pm 1.85
Control	59.5 \pm 1.17	66.2 \pm 1.06	74.5 \pm 0.79	79.1 \pm 1.07	86.7 \pm 1.24
Anti-BChE activity (% I)	26.6 \pm 1.11	43.7 \pm 1.67	58.6 \pm 1.16	64.6 \pm 1.88	70.5 \pm 1.11
Control	36.7 \pm 1.03	44.9 \pm 0.97	62.9 \pm 1.02	75.2 \pm 0.96	89.3 \pm 1.01

4. Conclusion

The replacement of synthetic with natural antioxidants and anticholinesterase may be valuable. The present study evaluates the antioxidant and anticholinesterase activity of absolute ethanolic turmeric crude extract. The results show that the extract exhibits strong antioxidant and anticholinesterase activity when verified with *in vitro* models. The results of *in vitro* studies suggest that ethanolic crude extract of Turmeric may be useful in defence against neurodegenerative disorders due to its anticholinesterase properties.

Conflict of interest

The authors declare no conflict of interest.

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