Antioxidant Activities of Raw and Nano Turmeric (*Curcuma longa* L.) Powders

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ABSTRACT

This study was performed to prepare nano turmeric powder (NTP) by ultrafine grinding from raw turmeric powder (RTP). In addition, evaluate and compare the antioxidant activity of RTP and NTP extracts with Butylated hydroxytoluene (BHT), Ascorbic acid and α-tocopherol. The results revealed that ultrafine grinding has effectively milling the turmeric particles to nano-scale. Prepared turmeric nanoparticles were spherical in shape with particle size range from 25-148 nm. The prepared NTP showed decrease in water holding capacity after the grinding treatment. On the other hand, the swelling capacity of NTP was higher than those of RTP. The results of antioxidant activity conducted on successive extracts showed significantly ($P \leq 0.05$) increasing as affected by ultrafine grinding. Results of antioxidant activity carried out according to the DPPH$^\bullet$ radical scavenging showed that free radical scavenging activity of NTP exhibited significantly ($P \leq 0.05$) stronger radical scavenging activity compared to RTP. The IC_{50} values of RTP and NTP were 21.45 and 7.82 (mg); respectively. Ultrafine grinding treatment significantly ($P \leq 0.05$) increased the radical scavenging abilities of NTP extracts on ABTS$^{\bullet+}$ radicals 739.26 mM TE at concentration of (5 mg/ml), compared to 311.18 mM TE. The IC_{50} values of RTP and NTP for ABTS$^{\bullet+}$ were 237.75 and 442.35 mM TE/g; respectively. It means NTP was considered a special natural material rich in polyphenols with a strong antioxidant function.
Keywords: Turmeric, curcumin, ultrafine grinding, nano-technology, functional properties and antioxidant activity.

INTRODUCTION

Antioxidants have received increasing attention due to their important role of delaying or preventing oxidative stress resulting from the generation reactive oxygen species. Antioxidants can be defined as substances which presence in very low relatively concentrations significantly inhibits the rate of oxidation. Gulcin *et al.,* 2004 reported that antioxidants have been widely used as food additive to provide protection against oxidative degradation of foods. Furthermore, the use of antioxidants as an adjunct to conventional or as an integral part of alternative cancer therapy is an area of intense research.

Spices are the natural antioxidant and antimicrobial agents most commonly used in foods. Addition of spices in foods not only imparts flavour and pungent stimuli but also provides antimicrobial property (Pandit *et al.,* 2011).

Turmeric (*Curcuma longa* L.) is a one of the most popular medicinal herbs, commercially used as a spices, pigment, flavour and preservative agent in curries and mustards, also utilized as a therapeutic agent used in several foods; because of its low side effects (Winter *et al.,* 2011 and Mahmood *et al.,* 2015). Turmeric have been containing natural antioxidants, and is reported to possess numerous medicinal properties and wide spectrum of biological actions which include; antioxidant, antiinflammatory, anticarcinogenic, antimutagenic, anticoagulant, antidiabetic, and antimicrobial activities (Ishitha *et al.,* 2004 and Sandhir *et al.,* 2015). Components of turmeric are named curcuminoids; these components are polyphenols exhibited strong antioxidant function that may be protect cells from the damage causes by unstable molecules known as free radicals (Anand *et al.,* 2010 and Prasanna *et al.,* 2011).

The research area of food technology still faces great challenges because these materials may confer unwanted properties to foods in terms of safety, processing, nutrition or acceptability by consumers. This is related to the intrinsic properties of the tissues or of their components. Although the turmeric has favorable multi functions, its applications are limited because their low water solubility, rapid intestines metabolite, slows dissolution rate and low bioavailability in vivo. So, the potency of antioxidants present in foods will depend not only on their levels in the foods but also on their bioavailability, that is, the extent to which the active forms of antioxidants are released from the food and absorbed through the gut (Rao, 2003; Prasanna *et al.,* 2011 and Sandhir *et al.,* 2015); there is therefore a need for finding alternative a processing technology that can efficiently produce new ingredients with optimized techno-functional and nutritional attributes.

Several strategies and different processing methods have been suggested, can be employed to modify the food product structure and composition to enhance the bioaccessibility of phenolic acids, such as using either wet-fractionation processes such as enzymatic treatments and fermentation (Moore *et al.,* 2006 and Penella *et al.,* 2008), or dry-fractionation processes such as ultra-fine grinding, air-classification and electrostatic separation (Hemery *et al.,* 2007 and Zaho *et al.,* 2009). Micron technology and nanotechnology are emerging technologies which show great potential in nutraceuticals and functional foods for human health improvement (Chen *et al.*, 2006). The greater surface area per mass unit compared with larger-sized particles of the same chemistry renders tiny particles more biological activity (Sanguansri and Augustin, 2006). Also, the possible uses of nanotechnology in food research has attracted much

attention, and become the focus of research in many countries (Zhu *et al.*, 2010).

Nanoparticle antioxidants include inorganic nanoparticles possessing major antioxidant properties, nanoparticles functionalized with antioxidants to function as an antioxidant delivery system. It is believed that nanoparticle antioxidants have strong and persistent interactions with biomolecules and would be more effective against free radical induced damage (Sandhir *et al.,* 2015 and and Amalraj *et al.,* 2016). The currently studies focus on the fabrication of various polymeric nanoformulations, such as nanofibers (Liu *et al.,* 2014), polymeric nanoparticles NPs (Xie *et al.,* 2015; Yoo *et al.,* 2015) and liposomes (Basak *et al.,* 2015).

Therefore, the aim of this investigation was to apply superfine grinding as one of modern techniques to modify the structure of tested turmeric powder that used as antioxidants in food products in Nanoparticle form; and compare between the antioxidant activity of prepared raw and nano turmeric powder.

MATERIALS AND METHODS

Materials:

Turmeric (*Curcuma longa* L.) powder was purchased during 2016 from local market, Cairo, Egypt. All chemicals and reagents used in the analytical methods (analytical grade) were produced by Sigma–Aldrich, Inc. (St. Louis, M., USA) and purchased from EL. Gomhouria trading chemicals and drugs company.

Methods:
Preparation of raw and nanoparticles turmeric powder:

Dried turmeric powder was passed through a 30 mesh sieve to prepare the raw turmeric powder (RTP) and packed in polyethylene bags and stored at -20 °C until use. The dried powder was ground to prepare the nano turmeric powder (NTP) using 5 mm zirconium oxide ball and zirconium oxide bowl volume 250 mL in a high energy nano ball milling (PQ-N2-100 Planetary Ball-mill, Retsch, Germany) as described by Zhu *et al.* (2010) with some modifications. Samples (100 g) were ground at 30 Hz frequency for 30 min at room temperature (~24 °C).

Particle size, zeta potential measurements and transmission electron microscopy:

Prepared turmeric nanoparticles (NTP) was examined by establishing their hydrodynamic diameter using Nano Zeta Sizer Instruments (Model Malvern, Nano Series, United Kingdom) according to Feng *et al.,* (2012); 4.5 mg of the prepared nanosuspension were solubilized in 10 mL deionized water then centrifuged at 5,500 rpm for 20 min. The average of five measurements and zeta potential were estimated on the basis of electrophoretic mobility under an electric field as an average of 30 measurements (Carvalho *et al.,* 2015). The surface morphology of nanoparticles and the particle size were analyzed with a JEOL JX 1230 technique with micro analyzer probe, Japan. This technique was used to determine the particle size of the investigated sample. The system was run by Nanoscope software.

Water holding capacity:

Water holding capacity (WHC) was determined according to the method developed by Zhang *et al.,* (2005). Centrifuge tubes were weighed (W). 0.5 gram (W1) of each examined sample and 7 mL distilled water were then poured into the tubes and incubated in a water bath at 60°C for 30 min followed by placed in cold water for 30 min. The tubes were centrifuged at 2683 g for 15 min thin, the supernatant was removed and the centrifuge tubes containing the sediment (W2) were weighed again. WHC was calculated as follows:

WHC (g/g wet sample) = (W2 – W)/W1.

Swelling capacity:

Swelling capacity (SC) was determined according to the method described by Lecumberri *et al.* (2007). One gram (M) of the samples was accurately weighed and poured into a 25mL calibrated cylinder. The initial volume (V1) was recorded. The samples were then mixed with 20 mL of distilled water and shacked manually for 5 min. Thereafter; they were placed into a water bath at 25°C for 24 h. The volumes of the samples (V2) were recorded again. Finally, SC was calculated using the following formula:

SC (mL/g wet sample) = (V2 –V1)/M.

Preparation of methanolic turmeric extracts:

Dried raw and nano turmeric powder samples (4 g) were homogenized for 1 min at 24,000 rpm using an Ultra-Turrax T-25 Tissue homogenizer (Janke & Kunkel, IKA®-Labortechnik, Saufen, Germany) in 50 mL of 80% methanol at room temperature. The homogenized extract was shaken overnight at 1,500 rpm. The extract was then centrifuged at 3,000 rpm for 15 min and filtered through 0.22 µm polyetetrafluorethylene (PTFE) filters (Carvalho *et al.,* 2015).

Determination of antioxidant activity of turmeric extracts:
Determination of antioxidant activity assay:

The antioxidant activity was examined by the conjugated diene method (Lingnert *et al.,* 1979). Each turmeric powder sample (5–25 mg/ml) in 2 g/l acetic acid solution (100 ml) was mixed with 2 ml of 10 mmol/l linoleic acid emulsion in 200 mmol/l sodium phosphate buffer (pH 6.5) in test tubes and placed in darkness at 37 °C to accelerate oxidation. After incubation for 15 h, 6 ml of 600 g/l methanol in deionized water was added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-1900 (UV/Vis.) spectrophotometer (Hitachi, Tokyo, Japan). The antioxidant activity was calculated as follows:

Antioxidant activity (%) = [(A_{234} of control - A_{234} of sample)/ A_{234} of control] X100.

A control consisted of methanol and the reagent solution without turmeric powder samples. An antioxidant activity value of 100% indicates the strongest antioxidant activity. IC_{50} value (mg/ml) is the effective concentration at which the antioxidant activity was 50% and was obtained by interpolation from linear regression analysis of plots where the abscissa represented the concentration of tested extracts and the

ordinate the average percent of antioxidant activity from three separate tests.

Determination of radical DPPH˙ scavenging activity:

The total radical scavenging capacity of the tested extracts was determined and compared to that of BHT, ascorbic acid and α-tocopherol by using the DPPH˙ scavenging methods. The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple colored methanol solution of the stable DPPH˙ radical. Free radical scavenging capacity of extracts were determined using the stable DPPH˙ according to Xie *et al.,* (2015). The final concentration was 200 µM for DPPH˙ and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min. Percent inhibition of the DPPH˙ free radical was calculated by the following equation:

Inhibition (%) = 100 × [(A$_{517}$ of control -A$_{517}$ of sample)/ A$_{517}$ of control]

Extract concentration of sample providing 50% inhibition (IC$_{50}$) was calculated using linear regression analysis.

Determination of radical ABTS˙⁺ scavenging activity:

ABTS˙⁺ also another forms a relatively stable free radical, which decolorizes in its non-radical form. The stock solutions of ABTS˙⁺ reagent was prepared according to Hwang and Do Thi (2014) by reacting equal quantities of a 7 mM aqueous solution of ABTS˙⁺ with 2.45 mM potassium persulfate for 16 h at room temperature (25°C) in the dark. The working solution was then prepared by diluting 1 mL ABTS˙⁺ solution with 60 mL of ethanol: water (50:50, v/v) to obtain an absorbance of 1.0± 0.02 units at 734 nm using the spectrophotometer. Extracts (50 µL) were allowed to react with 4.95 mL of the ABTS˙⁺ solution for 1 h in a dark condition. Then the absorbance was taken at 734 nm using the spectrophotometer. The standard curve was prepared using Trolox. Results were expressed as mM Trolox equivalents (TE)/g sample). Additional dilution was needed if the ABTS˙⁺ value measured was over the linear range of the standard.

Statistical Analysis:

All samples were analyzed in triplicates and the results were expressed as means ± standard division. Statistical analysis was assessed using the software SAS System for Windows (Statistical Analysis System, 2008). The significant difference between the mean values were determined by using the analysis of variance (ANOVA) and Duncan's multiple range test was conducted at a significance level of 95% (*P≤0.05*).

RESULTS AND DISCUSSION

Particle size, zeta potential measurements and transmission electron microscopy:

Dried turmeric powder was ground using high-energy nano-ball-milling; after ultrafine milling, the surface morphology of prepared turmeric nanoparticles and particle size were analyzed with Transmission Electron Microscopy (TEM) and dynamic light scattering (Nano Zeta Sizer). The TEM micrograph of prepared turmeric nanoparticles were shown in figure (1).
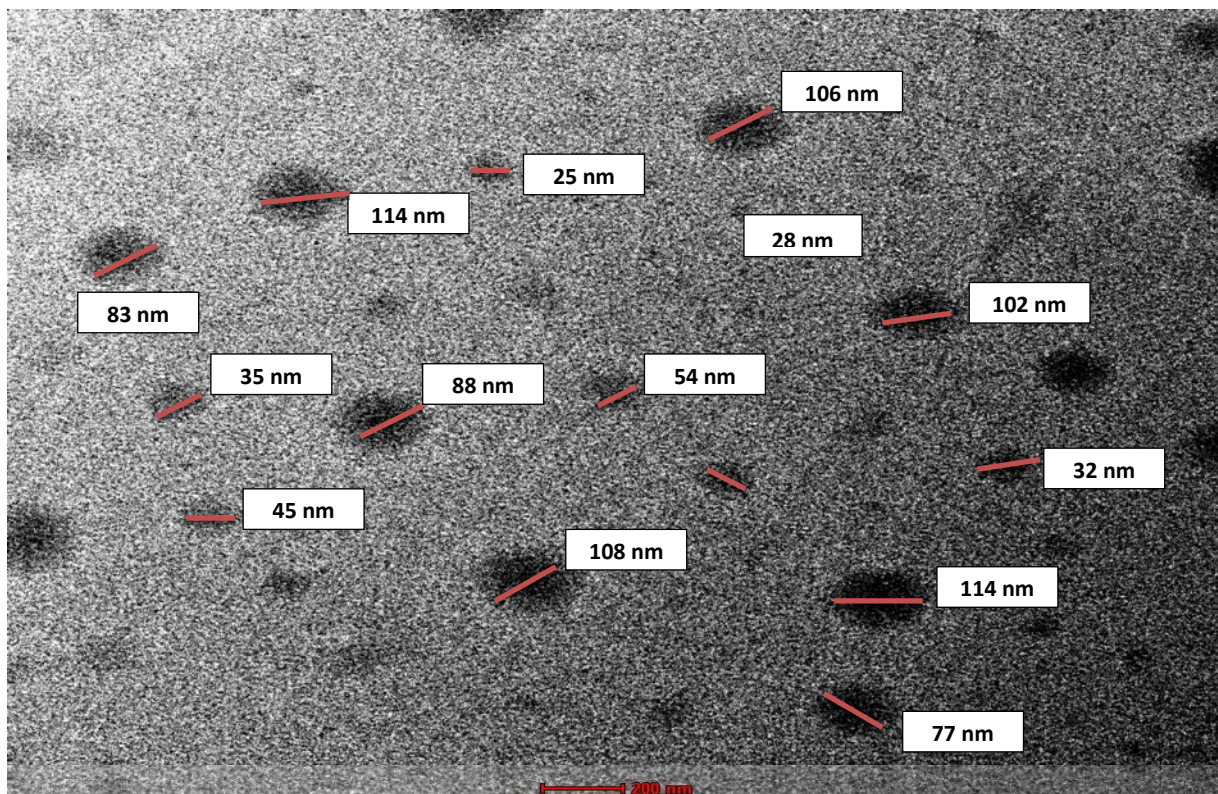

Fig. 1. Transmission electron microscopy micrograph of prepared turmeric nanoparticles.

The TEM image, gives a detailed view of the morphology and particle size of prepared turmeric nanoparticles. Nanoparticles were spherical in shape with particle size range from 28-148 nm, which indicated that they are in the nano-scale. The effect of ultrafine milling on prepared turmeric nanoparticles characteristics obtained by zeta sizer potential report indicated that, the hydrodynamic diameter analyses of the particles resulted in ranged from 85-164 nm.

Figure (2) shows a graph of particles distribution analysis using the Zeta sizer, it could be indicated that 100% of the particles (peak 1) presented mean diameter of 127.5 nm. The Polydispersity index (PDI) was 0.16; and zeta potential (4.7 mV) at the pH range of 4.5-6.9.
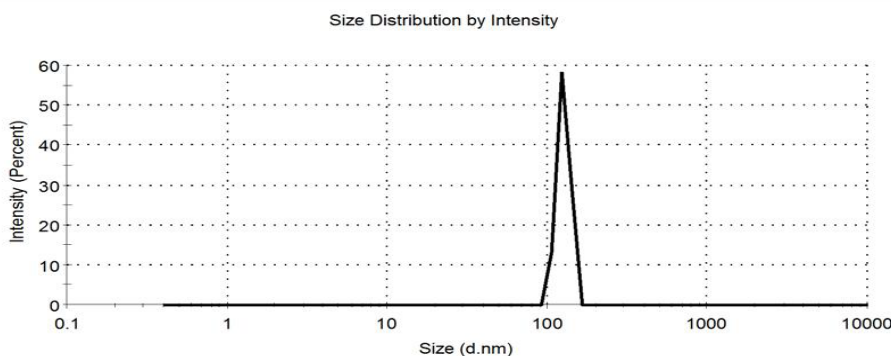
These results indicated that the milling process by high-energy ball-milling can effectively reduce the size of the particles to nano-scale; and it is thus feasible to utilize this treatment to prepared ultrafine powder as nanoparticles. The aforementioned results are in agreement with the results obtained by (Shaikh *et al.,* 2009 and Zhu *et al.,* 2010) who used ultrafine ball milling to decrease the particle size of curcumin and wheat bran by using multidimensional swing high-energy nano-ball-milling with ZrO_2 balls (6-10 mm in diameter). After ultrafine milling, the average of particle size was 344 and 264 nm for wheat bran and curcumin respectively.

Fig .2. Distribution of prepared turmeric nanoparticles size, gragh generated through zeta sizer instrument.

Hydration properties of raw and prepared nano turmeric powder:

Hydration properties of raw and prepared turmeric nanoparticles such as water holding capacity (WHC) and swelling capacity (SC) as affected by ultrafine grinding was measured and presented in figure (3).

WHC is the ability of a moist material to water when subjected to an external centrifugal gravity force or compression. It consists of the sum of bound water, hydrodynamic water and mainly physically trapped water (Vazquez-Ovando *et al.,* 2009). Raw turmeric powder (RTP) and nano turmeric powder (NTP) exhibited WHC values of 5.47 and 3.75 times its own weight, respectively.
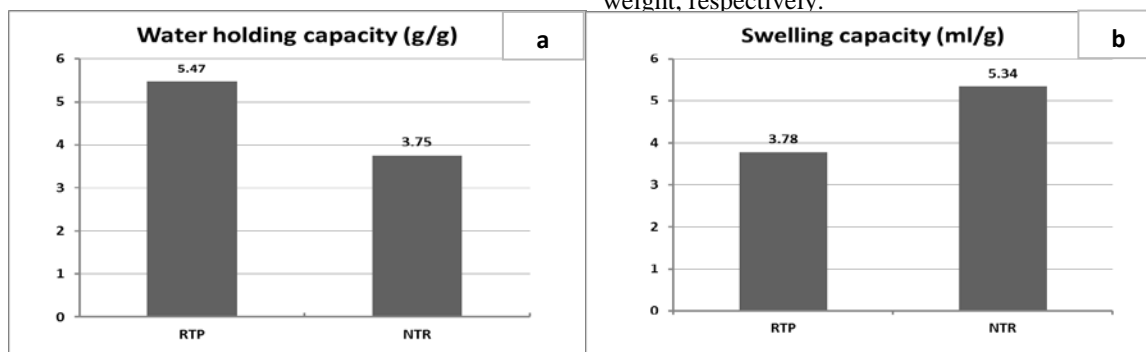

Fig .3. Hydration properties of raw and prepared nano turmeric powder as affected by ultrafine grinding.

The prepared turmeric nanoparticles powder showed decrease WHC after the grinding treatment. As shown in (fig. 3a), WHC value was reduced from 5.47 to 3.75 (g/g). Such effects could be attributed such as the reduction of particle size, also to altering of the fiber matrix structure. This could be explained according to (Kethireddipalli *et al.,* 2002 and Xie *et al.,* 2015) they reported that WHC depended on porous matrix structure formed by polysaccharide chains which can hold large amounts of water through hydrogen bond. Sangnark and Noomhorm (2003) and Sandhir *et al.,* (2015) established that, the particle size reduction of dietary fibers content has been associated with a lower ability to retain water and lower oil binding capacity.

On the other hand, the swelling capacity of nano turmeric powder (NTP) was higher than those of raw turmeric powder as shown in (fig. 3b). These obtained results indicated that the particle size of sample plays an important role in determining its hydration properties such as WHC and SC values.

Ming *et al.,* (2015) found that when average particle size decreased significantly, water holding capacity of the powders decreased in varied extents. However, swelling capacity increased.

Antioxidant activity of raw and prepared nano turmeric powder:

In this study the antioxidant activity was determined using various established *in vitro* indices

including antioxidant activity which determined by the conjugated diene method, DPPH$^\bullet$ radical scavenging activity, and ABTS$^{\bullet+}$ radical scavenging activity on successive extracts of raw and prepared nano turmeric powders as affected by ultrafine grinding:

Antioxidant activity by conjugated diene method:

The extracts of investigated samples were analyzed and compared using the conjugated diene method and the results are given in table (1). Results reveals that both of turmeric powders RTP and NTP showed moderate antioxidant activities (35.62 and

54.85%; respectively) at concentration 0.5% (5 mg/ml) compared with 65.14, 64.94 and 65.17% for BHT, ascorbic acid and α-tocopherol; respectively. As seen in the obtained data, it could be noticed that the antioxidant activity was increased with the increasing of the extract concentrations. NTP showed the highest antioxidant activities of 93.24% at 2.5% (25 mg/ml), while RTP showed lowest antioxidant activities of 63.89 at 2.5 % (25 mg/ml). In generally NTP exhibited significantly stronger antioxidant activity compared to RTP.

Table 1. Antioxidant activity of methanolic (RTP) and (NTP) extracts compared with BHT, ascorbic acid (AA) and α-tocopherol.

Concentrations (mg/ml)	Antioxidant Activity (%)				
	(RTP)	(NTP)	BHT	Ascorbic acid	α-tocopherol
5	35.62 [eE] ±0.11	54.85 [eD] ±0.08	65.14 [eB] ±0.11	64.94 [eC] ±0.15	65.17 [eA] ±0.07
10	42.72 [dE] ±0.05	71.56 [dD] ±0.17	81.39 [dA] ±0.21	80.19 [dC] ±0.03	80.95 [dB] ±0.06
15	51.91 [cE] ±0.03	80.15 [cD] ±0.13	88.05 [cA] ±0.09	85.18 [cC] ±0.04	86.83 [cB] ±0.07
20	60.66 [bE] ±0.22	84.73 [bD] ±0.17	93.82 [bA] ±0.16	89.76 [bC] ±0.11	92.19 [bB] ±0.14
25	63.89 [aE] ±0.19	93.24 [aC] ±0.15	95.78 [aA] ±0.20	92.45 [aD] ±0.04	94.83 [aB] ±0.10

- **Where: (RTP) Raw turmeric powder extract , (NTP) Nano turmeric powder extract**
- **Means of triplicate ± Standard Deviation (SD).**
- **Means followed by different small letters in the same column (effect of concentrations) are significantly by Duncan´s multiple test (P≤0.05).**
- **Means followed by different capital letters in the same raw (effect of Antioxidants) are significantly by Duncan´s multiple test (P≤0.05).**

DPPH$^\bullet$ radical scavenging activity:

DPPH$^\bullet$ radical scavenging method has been widely used to determine the free radical scavenging effectiveness of various antioxidant substances. In this assay, the antioxidants were able to reduce the stable radical DPPH$^\bullet$ to the yellow-colored which in form diphenyl-picrylhydrazine. The method is based on the reduction of DPPH$^\bullet$ in alcoholic solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction. DPPH$^\bullet$ is usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH$^\bullet$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Oyaizu, 1986).

The DPPH$^\bullet$ is stable free radical at room temperature; it is reduced in the presence of curcumin as main compound in turmeric, which decreases its coloring. The use of DPPH$^\bullet$ provides an easy, quick path to assess the antioxidant properties of curcumin (Kakran *et al.,* 2012).

Table (2) indicates the results of the analyses of antioxidant activity carried out according to the DPPH$^\bullet$ method, which requires the solubilization of the DPPH$^\bullet$ and the analyzed samples (RTP and NTP extracts) in methanol. The presented data showed that, the radical scavenging abilities of turmeric extracts (RTP and NTP) on DPPH$^\bullet$ radicals (39.75 and 61.22%, respectively) at 0.5% (5 mg/ml), and increased to (73.18 and 92.26%, respectively) at (25 mg/ml).

Table 2. DPPH$^\bullet$ radical scavenging activity of methanolic raw and nano turmeric powder extracts compared with BHT, ascorbic acid (AA) and α-tocopherol.

Concentrations (mg/ml)	DPPH$^\bullet$ radical scavenging activity (%)				
	(RTP)	(NTP)	BHT	Ascorbic acid	α-tocopherol
5	39.75 [dE] ±0.35	61.22 [eD] ±0.32	81.75 [eA] ±0.29	72.84 [eC] ±0.37	74.75 [dB] ±0.67
10	50.50 [cD] ±0.15	76.62 [dC] ±0.14	85.29 [dA] ±0.34	83.15 [dB] ±0.21	83.31 [cB] ±0.64
15	57.85 [bC] ±0.02	87.60 [cB] ±0.04	90.17 [cA] ±0.35	86.18 [cC] ±0.33	87.84 [bB] ±0.41
20	66.84 [aD] ±0.36	90.28 [bB] ±0.35	92.12 [bA] ±0.42	87.81 [bD] ±0.22	89.25 [ac] ±0.88
25	73.18 [aD] ±0.23	92.26 [aA] ±0.18	92.55 [aA] ±0.37	88.72 [aC] ±0.27	89.68 [aB] ±0.38

- **Means of triplicate ± Standard Deviation (SD).**
- **Means followed by different small letters in the same column (effect of concentrations) are significantly by Duncan´s multiple test (P≤0.05).**
- **Means followed by different capital letters in the same raw (effect of Antioxidants) are significantly by Duncan´s multiple test (P≤0.05).**

However, the obtained data indicated that the radical scavenging activity was increased with the increasing of the extract concentrations from (5 to 25 mg/ml). DPPH$^\bullet$ free radical scavenging activity of NTP exhibited significantly stronger radical scavenging activity compared to RTP. IC$_{50}$ is the required

concentration of sample antioxidants to scavenge 50% of DPPH$^\bullet$ radicals in the reaction mixtures under the experimental conditions, with the IC$_{50}$ value negatively associated the DPPH$^\bullet$ scavenging activity. IC$_{50}$ values of RTP and NTP were 21.45 and 7.82 (mg); respectively. The scavenging ability of turmeric could be attributed to

the donation of H from the β-diketone group of curcumin to DPPH$^•$ (Jovanovic *et al.,* 1999).

These results were in agreement with those of obtained by (Zaeoung *et al.,* 2005 and Xie *et al.,* 2015) they reported the strong antioxidant activity of methanol extract of turmeric against the DPPH$^•$ radical with % inhibition in the range of 86–92%.

Kakran *et al.* (2012) verified that the antioxidant activity studied through the elimination of DPPH$^•$ free radicals were higher for the turmeric nanoparticles than for the original turmeric powder. The authors attribute it to the higher solubility of nanoparticles and have probably proved antioxidant effect especially due to its phenolic hydroxyl groups. The active principle factor in turmeric is a group of phenolic compounds including curcumin which is very well known for its antioxidant activity (Miquel et al., 2002).

ABTS$^{•+}$ radical scavenging activity:

All the tested samples exhibited effectual radical scavenging activity ranged from 134.75 mM Trolox eq.(TE) to 742.55 mM TE/g, as seen in table (3). The presented data showed that, ultrafine grinding significantly increased the radical scavenging abilities of NTP extracts on ABTS$^{•+}$ radicals 739.26 mM TE at concentration of (25 mg/ml), compared to 311.18 mM TE. Also, the scavenging effect against ABTS$^{•+}$ at concentration (25 mg/ml) ranked the samples decreased in the order: BHT> NTP> α-tocopherol> ascorbic acid> RTP. The IC$_{50}$ values of RTP and NTP for ABTS$^{•+}$ were 237.75 and 442.35 mM TE/g; respectively. The inhibition by NTP of ABTS radical generation is higher than that by α-tocopherol, ascorbic acid and RTP, but lower than BHT.

Table 3. ABTS$^{•+}$ radical scavenging activity of methanolic raw and nano turmeric powder extracts compared with BHT, ascorbic acid (AA) and α-tocopherol.

Concentrations (mg/ml)	ABTS (mM Trolox eq.)				
	(RTP)	(NTP)	BHT	Ascorbic acid	α-tocopherol
5	134.75 ^{dE} ±0.85	268.25 ^{eD} ±0.56	381.75 ^{eA} ±0.73	362.84 ^{eC} ±0.51	364.75 ^{dB} ±0.79
10	194.50 ^{cD} ±0.45	387.62 ^{dC} ±0.67	455.29 ^{dA} ±0.38	420.15 ^{dB} ±0.63	419.31 ^{cB} ±0.59
15	265.85 ^{bD} ±0.28	484.60 ^{cB} ±0.45	565.17 ^{cA} ±0.57	446.18 ^{cC} ±0.48	487.84 ^{bB} ±0.56
20	307.84 ^{aE} ±0.38	620.28 ^{bB} ±0.58	672.12 ^{bA} ±0.72	537.81 ^{bD} ±0.68	589.25 ^{aC} ±0.68
25	311.18 ^{aE} ±0.48	739.26 ^{aA} ±0.48	742.55 ^{aA} ±0.54	638.72 ^{aC} ±0.69	659.68 ^{aB} ±0.49

- **Means of triplicate ± Standard Deviation (SD).**
- **Means followed by different small letters in the same column (effect of concentrations) are significantly by Duncan´s multiple test ($P≤0.05$).**
- **Means followed by different capital letters in the same raw (effect of Antioxidants) are significantly by Duncan´s multiple test ($P≤0.05$).**

Antioxidant mechanisms of turmeric have been studied by (Basak *et al.,* 2015; Panadit *et al.,* 2015 and Sandhir *et al.,* 2015), they established that phenolic antioxidants usually scavenge free radicals by an electron-transfer mechanism. The electron-donating ability is determined by the one electron oxidation potential of the parent antioxidants, expressed by definition as the reduction potential of the corresponding phenoxyl radicals.

CONCLUSION

The obtained results revealed that ultrafine grinding could effectively milling the turmeric particles to nano-scale. The results of antioxidant activity conducted on successive extracts showed significantly ($P≤0.05$) increasing as affected by ultrafine grinding. The antioxidant activity of NTP exhibited significantly ($P≤0.05$) stronger radical scavenging activity against both DPPH$^•$ and ABTS$^{•+}$ radicals. We can consider NTP a special natural material rich in polyphenols with a strong antioxidant function.

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النشاط المضاد للأكسدة للمساحيق الخام والنانو من الكركم
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إجريت هذه الدراسة لإعداد مساحيق في مدى النانو من الكركم بإستخدام تقنية الطحن متناهي الصغر من مسحوق الكركم الخام. بالإضافة لتقييم ومقارنة النشاط المضاد للأكسدة للمساحيق مع مضادات الأكسدة BHT وحمض الأسكوربيك والألفا توكوفيرول. وقد أظهرت النتائج فاعلية إستخدام تقنية الطحن متناهي الصغر في سحق جزيئات الكركم والحصول علي مساحيق في مدى النانو. وكان شكل جزيئات النانو المحضرة دائرى ويقع في مدى من 28-148 نانوميتر. كما أظهرت مساحيق النانو المحضرة إنخفاضاً في القدرة على ربط الماء بعد عملية الطحن وفي المقابل كان لها قدرة علي الإنتفاخ أعلي بالمقارنة بالصورة الخام للمساحيق. وأظهرت نتائج النشاط المضاد للأكسدة وجود زيادة معنوية في النشاط المضاد للأكسدة نتيجة لعملية الطحن. وقد دلت نتائج النشاط المضاد للأكسدة المعتمدة على كسح الشقوق الحرة DPPH• ، +•ABTS أن مساحيق النانو المحضرة أظهرت فروق وزيادة معنوية عن النشاط المضاد للأكسدة بكسح الشقوق الحرة لمساحيق الكركم الخام. وكانت قيم الـ IC_{50} (21.45 ، 7.82 مجم) لكل من المساحيق الخام ومساحيق النانو من الكركم على التوالي في كسح شقوق •DPPH. وكانت قيم الـ IC_{50} (237.75 ، 442.35 مللي مول لمكافي ترول وكس/ جرام) لكل من المساحيق الخام ومساحيق النانو من الكركم على التوالي في كسح شقوق +•ABTS . مما يعني أن مساحيق النانو المحضرة مواد طبيعية مميزة غنية بالبولي فينولات ولها نشاط قوي كمضاد للأكسدة.