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Physico-chemical Analysis, Total Polyphenolic Content and Antioxidant Capacity of Yellow Dye Extracted from *Curcuma longa*

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Abstract. Turmeric (*Curcuma longa*) is a well known condiment of the Asian cuisine and also used as ayurvedic medicine in the content, since ancient times due to its potential therapeutic properties. Main colouring constituent of *Curcuma longa* is curcumin and curcuminoids. In the present work natural yellow dye was extracted from rhizome of turmeric using an effective low cost method of solvent extraction. The developed natural yellow dye was assessed for physico-chemical analysis, toxicity, polyphenolic content and antioxidant activity. Physico-chemical assay showed good nutritional profile of the extracted natural yellow dye and quite safe at dose level 3.5g/Kg body wt. Significant phenolic content was found to be 63.32 mg GAE/100g, and also showed potent antioxidant capacity (% inhibition) ranging from 5.1-20.4 at 1-5 mg/mL concentration. Animal trials showed no mortality in the mice.

Keywords: *Curcuma longa*, curcumin, toxicity, phenolic contents, antioxidant activity

Introduction

Turmeric (*Curcumin longa* L), a member of the ginger family of herbs (*Zingiberaceae*), is a widely used spice that is native to the south of Asia. Most of the curcuma species grow in mountainous areas of the World, but some common species are often cultivated in gardens and used as a spice, food preservative and colouring, flavouring agent to the food and as medicinal plants (Zdrojewicz *et al*., 2017; Vyas, 2015). Curcumin is obtained from the dried rhizome of the plant *Curcuma longa*, first isolated almost two centuries ago and its structure was determined in 1910. Curcumin is the active ingredient of turmeric, which is used daily in Indian and other south Asian cuisines as a spice. Most commercial turmeric preparations consist of ~2-8% active curcumin (Choudhary and Sekhon, 2012). The main compounds in turmeric include curcumin (1E, 6E)-1, 7-Bis (4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3, 5-dione) and two curcuminoids, de-methoxycurcumin and bis-demethoxy curcumin (Fig. 1).

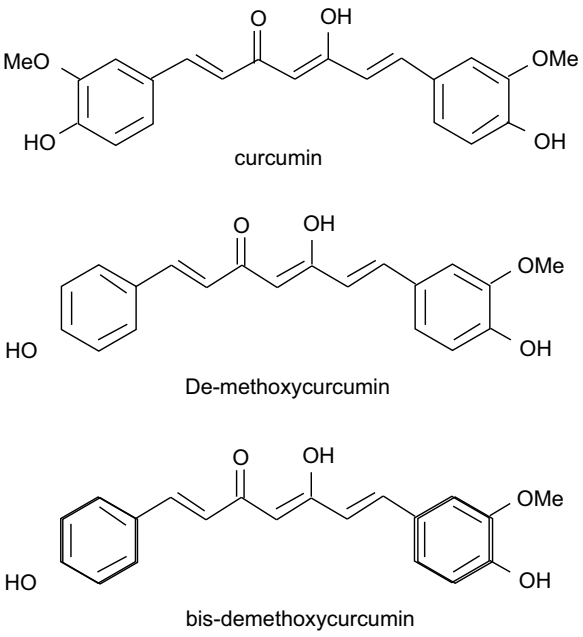

Fig. 1. Structures of curcumin and curcuminoids.

Curcumin and curcuminoids contribute the yellow colour to turmeric and have received increasing attention because of their many bio-activities. Current research shows that curcumin and curcuminoids have antifungal, anti-bacterial, anti-inflammatory, antioxidant, anti-mutagenicity and cholesterol lowering activities (Akter *et al*., 2019; Amalraj *et al*., 2017; and , 2017; *et al*., 2017). They can prevent rheumatoid arthritis in animal model (Funk *et al*., 2006). Oral administration of 5 and 10 mg/Kg curcumin significantly reduced the duration of immobility in depressive-like behaviours (tail suspension and forced swimming) in mice (Xu *et al*., 2005). Pre-treatment with curcumin significantly

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enhanced the rate of wound contraction, decreased mean wound healing time, increased synthesis of collagen, hexosamines, DNA and nitric oxide, and improved fibroblast and vascular densities (Jagetia *et al*., 2004).

Antioxidants are used in food industry to inhibit or delay the oxidation. Antioxidant found in plants are based upon constituent nutrients with demonstrated radical-scavenging capacities as well as upon non-vitamin or mineral substances. Plant based medicines contain flavonoids, polyphenols and flavoproteins which act as potent antioxidants like alpha-tocopherol, ascorbate and carotenoids (Kamble and Gacche, 2019). Further, some plants or specific combinations of herbs in formulations may act as antioxidants by exerting super-oxide scavenging activity or by increasing superoxide dismutase activity in various tissue sites Sawant *et al*., 2009). These groups of compounds are substances that may exert cell-protective action by more than one biochemical mechanism. In addition to antioxidant properties, cancer-protective factors are found in many plants including some fruits, vegetables and herbs (Alok *et al*., 2014).

Curcumin is main compound in turmeric and it act as free radical scavenger as well as hydrogen donor, binds with metals particularly iron and copper (Typek *et al*., 2019; Hatchera *et al*., 2012). Curcumin effectively inhibits intracellular amyloid toxicity at low dosages in rats due to its free radical scavenging activity (Ye and Zhang 2012). However, it is also effective in various models of antioxidant such as DPPH scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, ferric ions reducing power and ferrous ions chelating Ak and Gulcin (2008). The objectives of this research were physico-chemical analysis and to determine the total phenolic content (TPC) by using Folin-Ciocalteau method and antioxidant capacity of yellow dye extracted from turmeric by DPPH-free radical scavenging method.

Materials and Methods

Plant materials. Fresh plant turmeric (*Curcuma longa*) was purchased from local market of Lahore, Pakistan. Folin-Ciocalteu's (FC) phenol reagent was obtained from Merck (Darmstadt, Germany). Sodium carbonate, gallic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma (Steinheim, Germany).

Pigment extraction: Solvent extraction of curcumin was carried out using water. Solid liquid extraction was carried out by blending the sliced material with water using rhizome, solvent ratio of 1:5 (by mass per volume). The juice was then filtered to remove particulates. The filtrate was again used for further blending of the material. Slight acidification of the extraction medium done by addition of 0.15% ascorbic acid, 0.1% citric acid enhances curcumin stability. The filtrate was dried in hot air oven at 30-35 °C to form a dry product. The dry product was then ground using a rotating blade grinder to form a powder product that was passed through a 60 mesh sieve. After drying the residual weight was noted down and the amount of curcumin being extracted is calculated and then analyzed.

Physico-chemical analysis. Physico-chemical analysis like moisture, ash, fat fibre, protein contents were measured by AOAC methods (2016).

Animal studies /toxicological studies. Albino male mices weighing 25-30 g with ages of 2-3 month were used in this study and were fed with standard diet and water. They were kept in clean and dry cages and maintained in well ventilated animal house with 12 h light-12 h dark cycle. The animals were randomized into control and experimental groups and divided into 4 groups each group of 5 mice. Animals in group 1 were treated with distilled water. Animals in groups 2, 3 & 4 were treated with curcumin water extract 0.5, 1.5 and 3.5 g/Kg body wt.

Determination of total phenolic contents. Total phenolic contents of curcumin extracts were determined using Folin-Ciocalteu reagent as described by Singlaton and Rossi (1965). Turmeric extract (0.1 mL) were mixed thoroughly with 0.5 mL Folin-Ciocalteu reagent after 5 mins, 1.4 mL of 7.5% sodium carbonate (Na_2CO_3) was added and allowed to react for 90 min at room temperature. The absorbance was measured at 760 nm using spectrophotometer (UV-1700, Shimadzu Japan). Samples were measured in three replicates. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/100 g extract sample.

DPPH assay. The antioxidant activities of curcumin extracts were evaluated through free radical scavenging effect on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Akowuah *et al*. (2005). Three mL of 0.004% DPPH methanolic solution was added into 100 µL of sample extracts. The mixture was thoroughly mixed and kept in the dark for 30 min. The control was prepared using

3 mL of DPPH. The absorbance was measured at 517 nm using spectrophotometer (UV-1700, Shimadzu Japan). Samples were measured in three replicates. Percentage of DPPH scavenging activity was calculated as:

% inhibition of DPPH = [Abs control –(Abs sample/Abs control) × 100.

Statistical analysis. Experiment data were analyzed using Excel (Microsoft Inc.) and SPSS version 17.0 software. Significant differences between samples were analyzed using analysis of variance (ANOVA). Data obtained were reported as mean ± standard deviation (Steel *et al*., 1997).

Results and Discission

Unquestionably, the colour is a vital constituent of food. It is one of the rapid and indispensable factors depicting the quality and acceptability by the consumer. Generally artificial colours are added to improve the appearance of processed and preserved food products. Normally it has been appreciated that where natural colour of food is unattractive colouring matter may be added. In the present study natural yellow colour was extracted and assed for physico-chemical analysis, polyphenolic contents, toxicity and antioxidant activity.

Physico-chemical analysis of natural yellow dye. Table 1 shows the nutritional composition of the natural yellow dye. The basic nutritional compositions i.e. moisture, ash, protein, fat and total fiber were analyzed. The moisture and ash contents were found to be 11.76% and 1.83% respectively. Protein contents were 12.98% in yellow curcumin dye. 2.03% fat and 0.87% fiber contents were found in prepared yellow dye. The obtained nutritional analysis results suggest that the dye extracted from turmeric can be considered a potential dye to be used as natural colourant.

Table 1. Nutritional composition of the natural yellow dye

Parameters	Value (%)
Moisture	11.76 ± 0.8
Ash	1.83 ± 0.2
Protein	12.98 ± 0.9
Fat	2.03 ± 0.3
Fibre	0.87 ± 0.1

Data are presented ± SD

Toxicological studies. Toxicological studies were conducted on healthy mice to check the harmful effects of dye prepared. The oral dose of dye in pure drinking water was given in different quantities to animals and it was found that after 4 week the mice remained alive at dose 3.5 g/Kg body wt and also their weights were increased normally. It was found that there was no mortality and it is quite safe at said dose level.

Polyphenolic contents. Total phenolic contents of plants extract were tested using the diluted Folin-Ciocalteu reagent. Result clearly showed that curcumin had the total phenolic content with mean value of 63.32 mg GAE/100 g extract. Several studies (Wong *et al*., 2006; Wu *et al*., 2006; Shan *et al*., 2005) reported that phenolic compounds in spices and herbs significantly contributed to their antioxidant properties.

Antioxidant capacity. The antioxidant capacity of curcumin is attributed to its unique conjugated structure, which exists in an equilibrium between the diketo and keto-enol forms that are strongly favuored by intramolecular H-bonding Weber *et al*. (2005). Since de-methoxycurcumin and bis-demethoxycurcumin have similar structures like curcumin. The antioxidant capacity in term of % inhibition (DPPH) were range from 5.1-20.4 at 1-5 mg/mL concentration (Fig. 2). Curcumin shows typical radical-trapping ability as a chain-breaking antioxidant. Generally, the non-enzymatic antioxidant process of the phenolic material form the non-radical product (Tangkanakul *et al*., 2009; Chattopadhyay *et al*., 2004). Further studied by Masuda *et al*. (2001), the antioxidant mechanism of curcumin using linoleate as an oxidizable poly unsaturated lipid and proposed that the mechanism involved oxidative coupling reaction

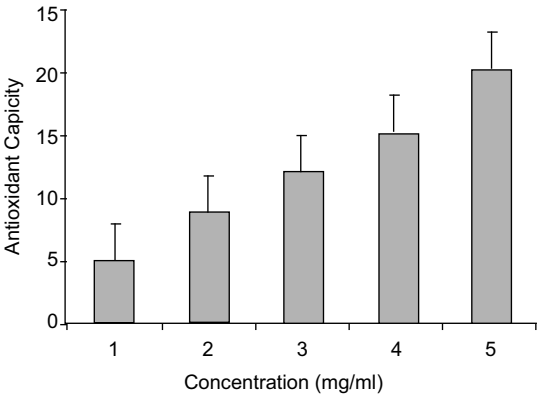

Fig. 2. Antioxidant capacity (% Inhibition) of curcumin.

at the 3 position of the curcumin with the lipid and a subsequent intramolecular Diels-Alder reaction.

Conclusion

In conclusion, it was found that the natural yellow dye (curcumin) was non toxic, nutritive and have potent free radical scavenging activity.

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Conflict of Interest. The authors declare no conflict of interest.

References

Ak, T., Gulçin, I. 2008. Antioxidant and radical scavenging properties of curcumin. Chemico-Biological Interactions, 174(1): 27-37.

Akowuah, G.A., Ismail, Z., Norhayati, I., Sadikun, A. 2005. The effects of different extraction solvents of varying polarities of polyphenols of Orthosiphon stamineus and evaluation of the free radical-scavenging. International Food Research Journal, 18: 529-534.

Akter, J., Hossain, M.A., Takara, K., Islam, M.Z., Hou, D. 2019. Antioxidant activity of different species and varieties of turmeric (Curcuma spp): Isolation of active compounds. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 215: 9-17.

Alok, S., Jain, S.K., Verma, A., Kumar, M., Mahor A., Sabharwal, M. 2014. Herbal antioxidant in clinical practice: A review. Asian Pacific Journal of Tropical Biomedicine, 4(1): 78-84.

Amalraj, A., Pius, A., Gopi, S. 2017. Biological activities of curcuminoids, other biomolecules from turmeric and their derivatives – A review. Journal of Traditional and Complementary Medicine, 7(2): 205-233.

AOAC. 2016. Association of Official Analytical Chemists. Methods of Analysis: 20th Ed. Washington, DC, USA.

Chattopadhyay, I., Biswas, K., Bandyopadhyay, U., Banerjee, R.K. 2004. Turmeric and curcumin: biological actions and medicinal applications. Current Science, 87(1): 44-53.

Choudhary, N., Sekhon, B.S. 2012. Potential therapeutic effect of curcumin-an update. Journal of Pharmaceutical Education and Research, 3(2): 64-71.

Erenler, R., Yilmaz, S., Aksit, H., Sen, O., Genc, N., Elmastas, M., Demirtas, I. 2009. Antioxidant activities of chemical constituents isolated from Echinops orientalis Trauv. Records of Natural Products, 8(1): 32-36.

Funk, J.L., Oyarzo, J.N., Frye, J.B., Chen, G., Lantz, R.C., Jolad, S.D., Solyom A.M., Timmermann, B.N. 2006. Turmeric extracts containing curcuminoids prevent experimental rheumatoid arthritis. Journal of Natural Products, 69: 351-355.

Hatchera, H., Planalpb, R., Chob, J., Tortia, F.M., Tortic, S.V. 2012. Curcumin: from ancient medicine to current clinical trials. Cell Molecular Life Science, 6(1): 36-45.

Hewlings, S.J., Kalman, D.S. 2017. Curcumin: A Review of Its' Effects on Human Health. Foods, 6(10): 92.

Jagetia, G.C., Rajanikant, G.K. 2004. Role of curcumin, a naturally occurring phenolic compound of turmeric in accelerating the repair of excision wound in mice whole body exposed to various doses of ?-radiation. Journal of Surgical Research, 120: 127-138.

Kamble, S.S., Gacche, R.N. 2019. Evaluation of anti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants. European Journal of Integrative Medicine, 25: 13-19.

Masuda, T., Maekawa, T., Hidaka, K., Bando, H., Takeda Y., Yamaguchi, H. 2001. Chemical studies on antioxidant mechanism of curcumin: analysis of oxidative coupling products from curcumin and linoleate. Journal of Agricultural and Food Chemistry, 49(5): 2539-2547.

Nelson, K.M., Dahlin, J.L., Bisson, J., Graham, J., Pauli, G.F., Walters, M.A. 2017. The Essential Medicinal Chemistry of Curcumin. Journal of Medicinal Chemistry, 60 (5):1620–1637.

Sawant, O., Kadam, V.J., Ghosh, R. 2009. In vitro free radical scavenging and antioxidant activity of Adiantum lunulatum. Journal of Herbal Medicine and Toxicology, 3(2): 39-44.

Shan, B., Cai, Y.Z., Sun, M., Corke, H. 2005. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. Journal of the Agricultural and Food Chemistry, 53: 7749–7759.

Singleton, V.L., Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic

acid reagents. American Journal of Enology and Viticulture, 16: 144-158.

Steel, R.G.D., Torrie, J.H., Dicky, D.A. 1997. Principles and Procedures of Statistics. A Biometrical Approach, 3rd Ed., McGraw Hill Book Co. Inc., New York.

Tangkanakul, P., Auttaviboonkul, P., Niyomwit, B., Lowvitoon, N., Maizura, M., Aminah, A., Wan Aida, W.M., Trakoontivakorn, G. 2009. Antioxidant capacity, total phenolic content and nutritional composition of Asian foods after thermal processing. International Food Research Journal, 16: 571-580.

Typek, R., Dawidowicz, A.L., Wianowska, D., Bernacik, K., Stankevic, M., Gil, M. 2019. Formation of aqueous and alcoholic adducts of curcumin during its extraction. Food Chemistry, 276: 101-109.

Vyas, K. 2015. The Cure is in the Roots: Turmeric. Journal of Nutritional Disorders and Therapy, 5:163.

Weber, W.M., Hunsaker, L.A., Abcouwer, S.F., Deck, L.M., Jagt, D.L.V. 2005. Anti-oxidant activities of curcumin and related enones. Bioorganic and Medicinal Chemistry, 13: 3811-3820.

Wong, C., Li, H., Cheng, K., Chen, F. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chemistry, 97: 705-711.

Wu, C.Q., Chen, F., Wang, X., Kim, H.J., He, G.Q., Haley-Zitlin V., Huang, G. 2006. Antioxidant constituents in feverfew (Tanacetum parthenium) extract and their chromatographic quantification. Food Chemistry, 96: 220–227.

Xu, Y., Ku, B., Yao, H., Lin, Y., Ma, X., Zhang, Y., Li, X. 2005. The effects of curcumin on depressive-like behaviors in mice. European Journal of Pharmacology, 518: 40-46.

Ye, J., Zhang, Y. 2012. Curcumin protects against intracellular amyloid toxicity in rat primary neurons. International Journal of Clinical and Experimental Medicine, 5(1): 44–49.

Zdrojewicz, Z., Szyca, M., Popowicz, E., Michalik, T., Smieszniak, B. Turmeric - not only spice. Polski Merkuriusz Lekarski. 2017. 42(252):227-230.