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## Antidiabetic and antioxidant potentials of spent turmeric oleoresin, a by-product from curcumin production industry

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## ABSTRACT

**Objective:** To investigate the antidiabetic and antioxidant activity of spent turmeric oleoresin (STO). **Methods:** Antidiabetic activity of STO evaluated by  $\alpha$ -amylase and  $\alpha$ -glucosidase enzyme inhibition assays. The antioxidant capacity studied by DPPH, ABTS, superoxide radical scavenging and metal chelating activity methods. **Results:** The STO showed good antidiabetic activity by inhibiting key enzymes linked to type 2 diabetes, viz  $\alpha$ -glucosidase and  $\alpha$ -amylase with an IC<sub>50</sub> values of 0.71 and 0.16  $\mu$ g/mL respectively. The IC<sub>50</sub> values for DPPH and ABTS assay were 58.1 and 33  $\mu$ g/mL respectively. STO effectively scavenged the superoxide free radical with an IC<sub>50</sub> value of 61.5  $\mu$ g/mL and showed a moderate iron chelation property. **Conclusions:** The above study reveals that the spent turmeric oleoresin being wasted at present can be used as antioxidant and antidiabetic agent in food and nutraceutical products.

### 1. Introduction

Turmeric (*Curcuma longa*) a rhizomatous herbaceous perennial plant of family Zingiberaceae, has been used in Indian and Chinese traditional medicine for various diseases, including biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders, and sinusitis [1,2,3,4]. Turmeric oleoresin (TO) contains 10–20% curcuminoids and a major portion of the TO prepared in the world has been used to isolate curcuminoids. The essential oil extracted from TO has been reported to have antibacterial [5], antioxidant and antifungal activities [6]. After the partial separation of curcuminoids, the remaining oleoresin (mother liquor) known as spent oleoresin (STO) is considered as an industrial waste and used as fuel in boilers. Previous studies showed that still some more amount of curcumin present in the STO and [7, 8].

Diabetes is a metabolic syndrome characterized by increase in blood glucose level. The impaired action or absolute deficiency of insulin results in imbalance of glucose metabolism and leads to diabetes mellitus. Oxidative stress, through the production of reactive oxygen species (ROS), reported as the root cause for the development of insulin resistance, beta-cell dysfunction, impaired glucose tolerance and type 2 diabetes mellitus [9].  $\alpha$ -glucosidase inhibitors such as acarbose and miglitol are being used in the treatment of type 2 diabetes because it prevents the degradation of complex carbohydrates into glucose. These synthetic drug molecules cause some side effects (flatulence, diarrhea and hepatitis). Recently, it has been shown that phenolic compounds play a role in mediating amylase inhibition and therefore have potential to contribute to the management of type 2 diabetes [10].

Turmeric and turmeric products have been widely prescribed for diabetic treatment in traditional methods. Hence the antioxidant and antidiabetic potentials of STO has to be evaluated to find application in food. The present study aims in utilizing STO effectively by assessing its ability

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to scavenge free radicals and the potential to inhibit the key enzymes linked to type 2 diabetes.

## 2. Materials and Methods

### 2.1. Chemicals, reagents and samples

Soluble starch, paranitrophenyl glucosidase (pNPG), ferrozine, nitrotetrazolium blue chloride (NBT), phenazinemethosulphate (PMS), gallic acid, Bradford's reagent, Bovine Serum Albumin (BSA),  $\alpha$ -amylase from *Aspergillus oryzae*,  $\alpha$ -glucosidase from Bakers Yeast, curcumin, acarbose, DPPH, ABTS, and quercetin were purchased from Sigma Aldrich (St. Louis, MO, USA). 3, 5 dinitrosalicylic acid (DNSA), EDTA, Folin-Ciocalteu's reagent, NADH and tris HCl were purchased from Sisco Research Laboratory Ltd, India. All other chemicals and reagents used were of analytical grade and obtained from Merck (Mumbai, India). The spent turmeric oleoresin (STO), after commercial isolation of curcuminoids from turmeric oleoresin, was procured from the local turmeric oleoresin industry from 3 different batches.

### 2.2. Evaluation of Antidiabetic activities

#### 2.2.1. $\alpha$ -amylase Inhibition Assay

The inhibition assay was performed according to the method of Prathapan et al [11] with minor modifications. 10  $\mu$  L of oleoresin/acarbose (0.025–0.125  $\mu$  g) in DMSO and 20  $\mu$  L of 0.02M sodium phosphate buffer (PH 6.9 with 0.006M NaCl) containing  $\alpha$ -amylase solution (0.5 mg/mL) were incubated at 25°C for 10 min. After pre-incubation, 25  $\mu$  L of 1% starch solution in 0.02M sodium phosphate buffer was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 50  $\mu$  L of dinitrosalicylic acid reagent. The tubes were then incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted with 500  $\mu$  L water and absorbance was measured at 540 nm using a multimode micro plate reader (Synergy 4, Biotek, USA).

#### 2.2.2. $\alpha$ -Glucosidase inhibition assay

This inhibition assay was performed according to the method of Shinde et al [12].  $\mu$ -glucosidase type I (20  $\mu$  L, 1 U/mL) was premixed with STO /acarbose and made up to 500  $\mu$  L with 50 mM phosphate buffer at PH 6.8. Then it was incubated for 5 min at 37°C. 1 mM pNPG (200  $\mu$  L) in 50mM of phosphate buffer was added to initiate the reaction and the mixture was further incubated for 20 min at 37°C. The reaction was terminated by the addition of 500  $\mu$  L of 1M sodium carbonate and the final volume was made up to 1.5 mL with water.  $\alpha$ -glucosidase activity of the mixtures was determined by measuring the quantity of nitrophenol released from pNPG. The absorbance of the mixtures at 405 nm was measured using a multimode microplate reader (Synergy 4, Biotek, USA). The concentration of STO required to inhibit 50% of  $\alpha$ -glucosidase under the assay conditions was defined as the IC50 value. The assay was

performed in triplicate and mean $\pm$ SD was reported.

### 2.3. Antioxidant Capacity Assays

#### 2.3.1. Determination of DPPH radical scavenging capacity

The antioxidant activity of STO was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method [13]. The 3mL reaction mixture contained 2.8mL of methanolic DPPH and 0.2mL of extract at various concentrations. In the control, methanol was used in the place of the sample. The contents were mixed well immediately and incubated for 30 min at room temperature (25–29°C). The degree of reduction of absorbance was recorded in a UV-Vis spectrophotometer (UV-2450PC, Shimadzu, Japan) at 517 nm.

The % of inhibition of free radicals was calculated using the following equation.

$$\% \text{ of inhibition} = [1 - (\text{Absorbance of Sample} / \text{absorbance of Control})] \times 100.$$

#### 2.3.2. Determination of Superoxide radical scavenging activity

Superoxide radical scavenging activity was measured using the method of Prathapan et al [14]. Superoxide anions were generated in a non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system through the reaction of PMS, NADH and oxygen. 250  $\mu$  L of NADH, 250  $\mu$  L NBT and 250  $\mu$  L PMS were added to varying concentrations of sample and standard. After standing for 5min at room temperature, the absorbance at 560 nm was measured (Synergy 4, Biotek, USA).

#### 2.3.3. Determination of ABTS radical scavenging activity

The assay was carried out using improved ABTS discoloration method [15]. The method is suitable for the study of both water-soluble and lipid-soluble antioxidants, pure compounds and food extracts. Different concentration of the standard (Trolox)/STO, ethanol and ABTS (900  $\mu$  l) were added in dark tubes to make the whole system to 1ml. The absorbance was measured spectrophotometrically at 734nm.

#### 2.3.4. Determination of metal chelating ability

The chelation of ferrous ions by the extract was estimated using ferrozine [16] with slight modifications and then compared with that of EDTA. Different concentrations of the extract were added to a solution of 1 mmol FeCl<sub>2</sub> (0.05 mL). The reaction was initiated by the addition of 1 mmol ferrozine (0.1 mL) and the mixture was finally made up to 1mL with methanol, mixed well and kept at room temperature for 10 min. After incubation, absorbance of the solution was measured spectrophotometrically at 562 nm.

### 2.4. Estimation of curcuminoids and TPC

#### 2.4.1. HPTLC quantification of curcuminoids

HPTLC was performed on 10 cm  $\times$  10 cm plates coated with 0.2-mm layers of silica gel 60F254 (Merck, Darmstadt, Germany). Samples and standards (approx. 5  $\mu$  L) were applied to the plates as 6mm wide bands, 5 mm apart, by means of

a CAMAG (Switzerland) Linomat V fitted with a Hamilton microliter syringe. The sample application rate was constant at  $0.1 \mu\text{L s}^{-1}$ . The TLC plates were developed using a Camag twin-trough glass tank which was pre-saturated with the mobile phase chloroform-methanol (95:5) for 1 hr under laboratory conditions of  $25 \pm 5^\circ\text{C}$  and 50 % relative humidity. After development, the plate was removed and dried and spots were visualized in UV light (UV cabinet, Camag, Switzerland). Data processing was performed with WinCATS planar chromatography manager software (version 1.4.3). The densitometric scanning was performed at a wave length 424 nm in absorption/reflection detection mode with a slit width of  $5 \times 0.45 \text{ mm}$ .

#### 2.4.2. Total Phenolic content (TPC)

The total phenolic content of the STO was determined using Folin-Ciocalteu reagent [17]. To 100 mL of different concentration of STO (three replicates), 500 mL of Folin-Ciocalteu reagent and 1 mL sodium carbonate (20%) were added and incubated at ambient temperature ( $25-270^\circ\text{C}$ ) for 90 min. The colour developed was measured at 760 nm using a UV-Vis spectrophotometer (UV-2450PC, Shimadzu, Japan). TPC was expressed as gallic acid equivalents.

#### 2.5. Statistical analysis

The experimental results were expressed as mean  $\pm$ SD (standard deviation) of triplicate measurements. The data were subjected to one-way analysis of variance (ANOVA) and the significance of differences between means was calculated by Duncan's multiple range test using SPSS for Windows, standard version 7.5.1, with the significance accepted at  $P < 0.05$ .

### 3. Results

#### 3.1. Antidiabetic efficacy

The ability of STO to inhibit  $\alpha$ -glucosidase and  $\alpha$ -amylase were evaluated using in-vitro model systems. The IC<sub>50</sub> values of the STO for the  $\alpha$ -glucosidase and  $\alpha$ -amylase assays were  $0.71 \pm 0.1$  and  $0.16 \pm 0.05$  corresponding standard (acarbose) got the values  $296 \pm 5$  and  $81 \pm 2 \mu\text{g/mL}$  respectively.

#### 3.2. Antioxidant Activity

The antioxidant activity of STO was evaluated by DPPH, Superoxide anion radical and ABTS radical scavenging studies and the IC<sub>50</sub> values of each assays were  $58.1 \pm 2.5$ ,  $61.5 \pm 0.9$  and  $33 \pm 0.7 \mu\text{g/mL}$  respectively. The corresponding standard (trolox) had got the values  $4.3 \pm 0.2$ ,  $7.8 \pm 0.3$  and  $530 \pm 7 \mu\text{g/mL}$ . For DPPH assay gallic acid and synthetic antioxidant (BHT) were also used as positive control, which had an IC<sub>50</sub> value of  $1.4 \pm 0.1$  and  $42 \pm 2 \mu\text{g/mL}$  respectively. The superoxide radical scavenging activity of BHT was found to be  $120 \pm 2 \mu\text{g/mL}$ .

#### 3.3. Metal chelating capacity

The ferrous ion chelating activities of STO were moderate

with an IC<sub>50</sub> value of  $1850 \pm 15 \mu\text{g/mL}$ . The method is based on the principle that ferrozine can quantitatively form complex with Fe<sup>2+</sup>. In the presence of chelating agents, the complex formation is disrupted, which result that the red colour of the complex is decreased.

#### 3.4. Curcuminoids and TPC in STO

Curcuminoids and TPC in STO were estimated by HPTLC and UV spectrophotometric analysis. It showed that 11.2% of curcuminoids was present in STO with 5.8% curcumin, 2.8% demethoxycurcumin and 2.7% bisdemethoxy curcumin. The % of total phenolic content in STO was found to be 20.1%.

### 4. Discussion

Spent turmeric oleoresin which is being used as an industrial waste, showed good antidiabetic activity by inhibiting the key enzymes linked to type 2 diabetes. STO exhibited potent  $\alpha$ -glucosidase and amylase inhibitory efficacy in a dose dependent manner. Results indicating that STO inhibited the glucosidase and amylase enzymes more effectively than the reference standard. The antidiabetic property of STO is reported for the first time by this experiment.

DPPH is one of the powerful free radical which is used to evaluate the electron donating capacity of antioxidants [18]. The STO was able to reduce the stable pink colored free radical DPPH to yellow colored diphenyl picrylhydrazine. The estimated IC<sub>50</sub> values stands for the concentration of antioxidant (sample) required to scavenge 50% of radicals in the reaction mixture and are inversely related to their antioxidant activity. The results showed that free radical scavenging activity of STO was almost close to synthetic antioxidant (BHT) and lower than gallic acid. Hence it is possible to reduce the use of carcinogenic synthetic antioxidant BHT with STO.

Superoxide radical scavenging activity is one of the most important ways of clarifying the mechanisms of antioxidant activity of STO since superoxide can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, and it is very harmful to cellular components in the biological system. The activity of STO was almost double than that of standard BHT ( $120 \pm 2 \mu\text{g/mL}$ ) under the experimental condition, this supports the antioxidant potential of STO.

ABTS is another powerful free radical which is used to evaluate the electron donating capacity of antioxidants. STO significantly scavenges the ABTS radicals even though its activity is comparatively lower than that of standard trolox (IC<sub>50</sub>  $7.8 \pm 0.3 \mu\text{g/mL}$ ). The free radical scavenging property of STO also helps to control and reduce the after effects caused by the diabetes, since free radicals are the cause of many degenerative diseases including diabetes.

Considering metal chelating activity, the formation of Fe<sup>2+</sup>-ferrozine complex was prevented by STO, which was evidence of the same. Transition metals have been proposed to catalyse the initiation and propagation of radical chain reaction in lipid peroxidation. STO could chelate ferrous ions even though its

activity is less than EDTA. So the metal chelating study of STO has some significance.

The antidiabetic property of STO was due to the presence of curcuminoids and the turmeric essential oil in it. The effect of curcumin on blood glucose level has been previously reported. In addition it is reported that curcuminoids and ar-turmerone shows blood glucose lowering activity [19]. The % of total phenolic content in STO was 20.1%. It is known that the phenolic compounds contribute to overall antioxidant potential of plant materials. The potent antioxidant activity exhibited by STO was due to the presence phenolic compounds; particularly curcuminoids (11.2%). STO also contains glycerol, malic acid, citric acid, fumaric acid, ferulic acid, coumaric acid, vanillic acid and other polar metabolites [20]. The volatile oil content was found to be 35%; the major component was ar-turmerone. The pronounced antidiabetic and antioxidant activity of STO might be due to the additive or synergistic action of curcuminoids, volatile oils and other constituents. These positive results provide wide scope for the utilization of spent turmeric oleoresin as natural antioxidant/antidiabetic agent, preservative and also colorant in food, nutraceutical and pharmaceutical formulations.

### Conflict of interest statement

We declare that we have no conflict of interest.

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