

Phytochemical analysis and biological activities of ethanolic extract of *Curcuma longa* rhizome

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(With 3 Figures)

Abstract

Curcuma longa is an important dietary plant which possess several pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer and anti clotting etc. The aim of the present study was to determine the phenolic profile of *Curcuma longa* and *in vitro* antioxidant and antidiabetic activities. In HPLC chromatogram of *Curcuma longa* rhizome extract 15 phenolic compounds were identified namely Digalloyl-hexoside, Caffeic acid hexoside, Curdione, Coumaric, Caffeic acid, Sinapic acid, Quercetin-3-D-galactoside, Casuarinin, Bisdemethoxycurcumin, Curcuminol, Demethoxycurcumin, and Isorhamnetin, Valoneic acid bilactone, Curcumin, Curcumin-O-glucuronide respectively. The ethanolic extract displayed an IC₅₀ value of 37.1±0.3 µg/ml against alpha glucosidase. The IC₅₀ value of DPPH radical scavenging activity was 27.2 ± 1.1 µg/mL. It is concluded that ethanolic extract of *Curcuma longa* is rich source of curcumin and contain several important phenolics. The *in vitro* antioxidant and alpha glucosidase inhibitory effect of the plant justifies its popular use in traditional medicine.

Key words: *Curcuma longa*, HPLC analysis, Curcuminoid, DPPH activity, alpha glucosidase inhibitory activity.

Análise fitoquímica e atividades biológicas do extrato etanólico do rizoma de *Curcuma longa*

Resumo

A *Curcuma longa* é uma importante planta presente na dieta da população, pois possui diversas atividades farmacológicas, incluindo antioxidante, antimicrobiana, anti-inflamatória, anticancerígena, anticoagulante etc. O objetivo do presente estudo foi elucidar o perfil fenólico da *Curcuma longa* e determinar as atividades antioxidante e antidiabética *in vitro* do extrato. No cromatograma por HPLC do extrato de rizoma de *Curcuma longa*, foram identificados 15 compostos fenólicos: digaloil-hexosídeo, hexosídeo de ácido cafeico, curdiona, cumárico, ácido cafeico, ácido sinápico, quercetina-3-D-galactosídeo, casuarinina, bisdemetoxicurcumina, curcuminol, demetoxicurcumina, isoramnetina, bilactona de ácido valônico, curcumina e curcumina-O-glicuronídeo. O extrato etanólico apresentou um valor de IC₅₀ de 37,1 ± 0,3 µg / mL em relação à alfa-glucosidase. O valor de IC₅₀ da atividade de eliminação de radicais DPPH foi de 27,2 ± 1,1 µg / mL. Conclui-se que o extrato etanólico de *Curcuma longa* é uma rica fonte de curcumina e contém várias substâncias fenólicas importantes. O efeito antioxidante *in vitro* e inibidor da alfa-glucosidase da planta justifica seu uso popular na medicina tradicional.

Palavras-chave: *Curcuma longa*, análise por HPLC, Curcuminóide, atividade DPPH, atividade inibidora de alfa-glucosidase.

1. Introduction

Diabetes mellitus (DM) is the disorder with servere micro and macro complication that results in significant deaths. It is main causes of death in the world. There are limited effective therapies to cure diabetes. The use of insulin and other antidiabetic agents cause unpleasant side effects, thus there is need to find safe natural products to

treat diabetes. Long term complications such as organs failure are the result of chronic hyperglycemia of diabetes (Olatunde et al., 2014). DM also represented by lipidaemia and oxidative stress (Ghazanfar et al., 2014). Although many traditional medicinal plants are effective in decreasing blood sugar, most of these are not practically utilized in severe

diabetes (Ranilla et al., 2010). Many plants have shown biological activities and utilized as standardized extracts (Dar et al., 2019; Silva et al., 2019; Pontes et al., 2019).

Turmeric (*Curcuma longa*) is a common plant which belongs to family Zingiberaceae (Thomas-Eapen, 2009). The rhizome of the plant are dried, ground and boiled to get yellow powder which is used as food color in curry powder in Asian Countries (Goel et al., 2008). Turmeric powder is a food preservative due to its antioxidant action and adds to the flavor and fragrance of food (El Demerdash et al., 2012; Aggarwal et al., 2007). Turmeric contains curcumin, demethoxycurcumin, bisdemethoxycurcumin and rich in volatile oil (Shehzad et al., 2013). *Curcuma longa* possess several pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, anticancer, anti clotting etc. (Akbik et al., 2014; Aggarwal and Harikumar, 2009; Widowati et al., 2018). The phenolic composition of *Cucuma longa* has not been studied in detail. The present study was therefore, aimed to evaluate the phenolic composition of ethanol extract of *Curcuma longa*. The antioxidant and antidiabetic activities of *Curcuma longa* were also determined.

2. Materials and Methods

2.1. Plant extract

Rhizome of the plant was collected locally, identified by a botanist and a voucher specimen was submitted at the Herbarium of the University of Poonch, Department of Botany (Ref. No. BOT/2018/35).

Finely grounded rhizome (25g) was soaked for 3 days in ethanol (500 ml) with constant stirring and filtered with the help of filter paper. The extraction procedure was repeated three times and the filtrates were pooled. The filtrate was evaporated by rotary evaporator (45 °C) producing 3.5g (14% w/w) extract.

2.2. HPLC analysis of phenolic compounds

The dried ethnaol extract of *Curcuma longa* was dissolved in ethanol (1 mg/mL), filtered and subjected for analysis by Shimadzu HPLC system as reported by Zeb (2015). The best separation was achieved in 40 min using gradient elution of methanol, deionized water and acetic acid on a Zorbax plus C18 column (4.6 × 100 mm, 3.5 µm) at 25 °C. The chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–500 nm).

2.3. Determination of alpha glucosidase inhibitory activity of extract

Alpha glucosidase inhibitory activity was determined by the method described by Sancheti et al., (2011).

2.4. DPPH radical scavenging activity of extract

Scavenging of the DPPH radical (ethanolic solution of 0.25 mM) was assayed *in vitro* (Hatano et al., 1988). The results were expressed as percent inhibition calculated from the control.

2.5. Statistical analysis

The results were expressed as mean ± standard deviation. The data was analyzed by ONE WAY ANNOVA followed by Duncan multiple range test (DMRT) where necessary. Satista 7.1 was used as software package.

3. Results and Discussion

In HPLC chromatogram of *Curcuma longa* rhizome extract the peak of Digalloyl-hexoside appeared at retention time of 1 min (peak 1), Caffeic acid hexoside at 8.6 min (peak 2), Curdione at 10.3 min (peak 3), Coumaric acid at 13.1 min (peak 4), Caffeic acid at 14.1 min (peak 5), Sinapic acid at 15.9 min (peak 6), Qurecetin-3-D-galactoside at 23.6 min (peak 7), Casuarinin at 25.2 min (peak 8), Bisdemethoxycurcumin at 26.1 (peak 9), Curcuminol at 26.7 min (peak 10), Demethoxycurcumin at 29.6 min (peak 11) and Isorhamnetin at 30.1 min (peak 12), Valoneic acid bilactone at 31 min (peak 13), Curcumin at 35 min (peak 14), curcumin-*O*-glucuronide at 37.2 min (peak 15) respectively (Figure 1 and Table 1). Plants are rich in phenolic compounds which stabilize the free radicals by inhibiting lipid peroxidation (Newairy and Abdou, 2009; Juang et al., 2004). The pure curcumin was detected at t_R of 35 min with a concentration of 3202.9 µg/g in dry ethanolic extract of rhizome. Demethoxycurcumin concentration was noted to be 2313.9 µg/g while, Bisdemethoxycurcumin amount was found to 250.1 µg/g. The previous studies have shown the presence of three compounds, namely curcumin (60-80%), demethoxycurcumin (15-30%) and bisdesmethoxycurcumin (2-6%) (Ravindranath and Satyanarayana, 1980, Satyavati et al., 1976).

The inhibition of alpha glucosidase activity was observed at a concentration range of 10-250 µg/ml and

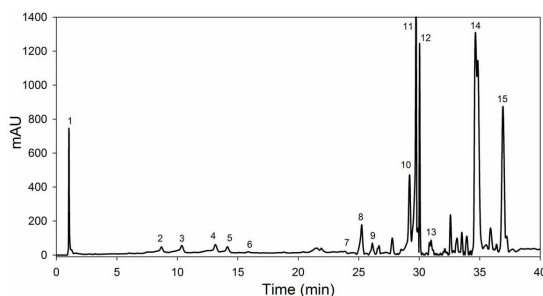
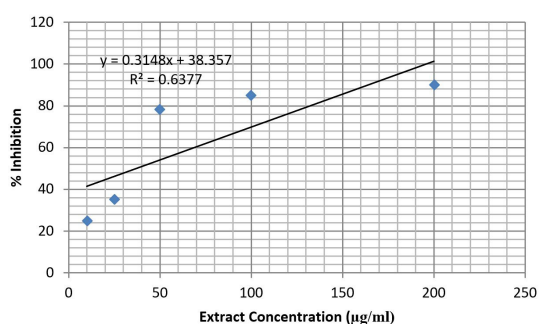
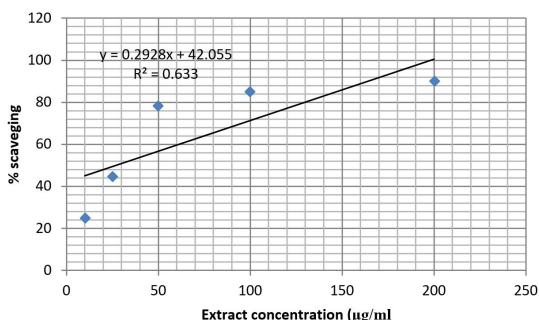


Figure 1. Representative high performance liquid chromatography profile of *Curcuma longa* rhizome. Digalloyl-hexoside appeared at retention time of 1 min (peak 1), Caffeic acid hexoside at 8.6 min (peak 2), Curdione at 10.3 min (peak 3), Coumaric acid at 13.1 min (peak 4), Caffeic acid at 14.1 min (peak 5), Sinapic acid at 15.9 min (peak 6), Qurecetin-3-D-galactoside at 23.6 min (peak 7), Casuarinin at 25.2 min (peak 8), Bisdemethoxycurcumin at 26.1 (peak 9), Curcuminol at 26.7 min (peak 10), Demethoxycurcumin at 29.6 min (peak 11) and Isorhamnetin at 30.1 min (peak 12), Valoneic acid bilactone at 31 min (peak 13), Curcumin at 35 min (peak 14), curcumin-*O*-glucuronide at 37.2 min (peak 15).

Table 1. Identification and quantification of phenolic compounds in *Curcuma longa*

Peak	Rt	Identity	Absorption spectra	Concentration (µg/g)
1	1	Digalloyl-hexoside	364, 235	392.3±5.6
2	8.6	Caffeic acid hexoside	283, 232	157.4±4.1
3	10.3	Curdione	303, 280	213.3±3.9
4	13.1	Coumaric acid	310, 290sh, 228	126.6±8.5
5	14.1	Caffeic acid	323, 296sh, 228	64.0±4.4
6	15.9	Sinapic acid	323, 288, 228	13.7±2.1
7	23.6	Qurecetin-3-D-galactoside	356, 256	9.8±1.8
8	25.2	Casuarinin	367, 266	591.9±6.5
9	26.1	Bisdemethoxycurcumin	418, 250	250.1±7.3
10	26.7	Curcuminol	426, 284	180.6±2.7
11	29.6	Demethoxycurcumin	420, 280	2,313.9±12.6
12	30.1	Isorhamnetin	374, 250	1,767.7±14.5
13	31	Valoneic acid bilactone	373, 265	1,081.9±9.7
14	35	Curcumin	428, 264	3,202.9±24.5
15	37.2	curcumin- <i>O</i> -glucuronide	426, 270	2,151.6±12.1

**Figure 2.** Glucosidase inhibitory activity by ethanolic extract of *Curcuma longa*. Values are means±SD (n=3). Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.**Figure 3.** DPPH radical scavenging activity of ethanolic extract obtained from rhizome of *Curcuma longa*. Values are means±SD (n=3). Values in figures which share different letters are significantly ($p<0.05$) different from each other by DMRT.

increased with increasing concentration of extract which indicates that extract possess *in vitro* antidiabetic activity (Figure 2). The IC_{50} for inhibition of alpha glucosidase was 37.1 ± 0.3 µg/ml for ethanolic extract obtained from rhizome.

Blood glucose is elevated when carbohydrate rich diet is consumed as the complex carbohydrate is rapidly absorbed in human intestine due to action α -glucosidase enzyme which breaks disaccharides into absorbable monosaccharides. The inhibitors of α -glucosidase inhibits the digestion of disaccharides and enable overall smooth glucose profile (Casirola and Ferraris, 2006). The natural products have great diversity in their structure and are potential inhibitor of alpha glucosidase. The phenolic rich ethanolic extract of *Curcuma longa* has higher potential to inhibit alpha glucosidase and thus can be effectively utilized in diabetes.

The antioxidant activity of the extract was tested by widely used DPPH method. The ethanolic solution of DPPH free radical is reduced on treatment with antioxidants present in the extract. In the DPPH assay, *Curcuma longa* showed excellent scavenging against the radical (Figure 3). The activity was the highest at maximum concentration, with an IC_{50} value of 27.2 ± 1.1 µg/mL ($r^2 = 0.96$). The pure gallic acid showed an IC_{50} value of 3.1 ± 0.7 µg/mL ($r^2 = 0.99$). Antioxidants act at different stages (prevention, interception, and repair) and by different mechanisms as reducing agents by donating hydrogen, by quenching of singlet oxygen, and by acting as chelators and trapping free radicals (Devasagayam et al., 2004). The high DPPH radical scavenging activity of *Curcuma longa* suggests use against diseases arising from free radical attack.

4. Conclusions

The results showed that *Curcuma longa* rhizome can be considered as potential source of substances with anti-oxidant and anti-diabetic activities. The findings suggest research continuity with the fractions of the crude extract in oxidative stress and other degenerative diseases. These results clearly demonstrated that *Curcuma longa* has the potential to be selected as an alternative medicinal and food plant that can be utilized in health food products, functional tea and pharmaceutical products.

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