Phytopreventive Antihyperlipidemic Activity of Curcuma Zedoaria

A.R. Srividya1*, S.P. Dhanabal2, Ajit Kumar Yadav3, M.N. Sathish Kumar4 and V.J. Vishnuvarthan1

1Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ooty-643 001, Tamil Nadu, India
2Department of Phytopharmacy and Phytochemistry, JSS College of Pharmacy, Ooty-643 001, Tamil Nadu, India
3Department of Pharmaceutical Biotechnology, Invertis Institute of Pharmacy, Bareilly-243 123, Uttar Pradesh, India
4Department of Pharmacology, JSS College of Pharmacy, Ooty-643 001, Tamil Nadu, India

*E-mail: pharmarsrividya@yahoo.com, ajit.y@invertis.org
Tel.: +91-9484175648, +91-423-2443393

Received: November 11, 2011 / Revised: April 10, 2012 / Accepted: April 11, 2012

Curcuma zedoaria belongs to the family Zingeberaceae. Hydroethanolic extract of Curcuma zedoaria rhizome showed only the presence of alkaloids. Total phenol content was found to be 34.45±1.9 expressed as mg/g equivalent of gallic acid. Total flavonol content was found to be 45.56±2.38 mg/g equivalent of quercetin. In vitro antioxidant activity IC50 value for hydroethanolic extract was found to be 930±16.35 for DPPH method, >1000 µg/ml for Nitric oxide method. Concentration required for reducing power was found to be 2.525±0.023 µg/ml. total antioxidant capacity was found to be 230.2±1.32 which was expressed as mM equivalent of Ascorbic acid. The extract was found to be effective in reducing TC levels after 12 days of pre-treatment with extract at a dose of 200 and 400 mg/kg b/w reduced by 17.1% and 19.65%. No significant changes were seen on LDL, VLDL and HDL cholesterol levels.

Key words: Curcuma zedoaria, Antihyperlipidemic, Antioxidant, Zingeberaceae, Lipid profile.

INTRODUCTION
Natural products are associated with a wide range of bioactivities and have played a vital role in treatment of diseases (Dahiya and Gautam, 2011; Jain et al 2011; Zahid Hosen et al 2011). Curcuma zedoaria is a perennial herb which is cultivated throughout India and traditionally, used as carminative, stomachic, gastrointestinal stimulant, diuretic, expectorant, demulcent, rubefacient as well as used in flatulence (Riaz et al 2011; Kim et al 2000). Zedoary's effect on digestive system is similar to ginger but milder. The Ayurvedic pharmacopoeia indicated use of rhizome in goiter. These rhizomes found to contain a number of terpenoids, including curcumene, curcumeneone, curdione, curcumenol, curzeronene epoxide, a volatile oil (1.0-1.5%) resembling ginger oil and starch (50%). Traditionally, this rhizome is used for the treatment of goiter and as antitumor, anti allergic and antimicrobial (Figure 1). In present work, antihyperlipidemic activity of the herb Curcuma zedoaria is reported.

Fig. 1. Curcuma zedoaria rhizome
**MATERIALS AND METHODS**

**Collection and authentication**

_Curcuma zedoaria_ rhizome was purchased from the PSS Herbs Pvt. Ltd, Kerala, India and authenticated by Dr. S. Rajan, botanist, Nilgiris. The rhizome was shade dried, milled and coarse powder was separated. The different extracts were prepared and percentage yield of the extract was calculated.

**Qualitative phytochemical screening**

A systematic and complete study of crude drug including a complete investigation on both primary and secondary metabolite derived from plant metabolism was carried out. The extracts prepared were tested for qualitative chemical tests for the identification of various phytoconstituents. (Kokate, 2003; Raman, 2006). Dried _Curcuma zedoaria_ rhizome was powdered and macerated separately with 50% ethanol by cold maceration process.

**Quantitative phytochemical analysis**

Estimation of total phenolic content was performed by Folin-Ciocalteu reagent (Mori, 1988; Sundararajan et al 2006). Estimation of total flavonoids content were performed by AlCl₃ method (Mori, 1988; Kaufman, 1999; Mills, 2000).

**Biological studies**

_In vitro_ antioxidant activity was performed by the DPPH radical scavenging method, nitric oxide radical inhibition activity (Srinivasan et al 2007), reducing power (Liu et al 2008), total antioxidant capacity (El-Beshbissy et al 2006).

**Antihyperlipidemic activity**

Healthy adult male rats weighing 150-220 g were obtained from animal house of JSS College of Pharmacy, Ooty, India. The animal house was well ventilated and these animals had 12±1 h day and night schedule with the temperature between 20±2°C. The animals were housed in large spacious hygienic cages during the course of experimental study. Hyperlipidemia was induced by poloxamer (1 gm/kg b/w) administered intraperitoneally.

**Experimental design:**

The animals were divided into 5 groups of six animals each and fed orally. Group-I: Vehicle control; Group-II: Hyperlipidemic control (Poloxamer 1 gm/kg body wt.); Group-III: Positive control (Atorvastatin 75 mg/kg); Group-IV: Hydroethanolic extract of _Curcuma zedoaria_ (200 mg/kg body wt.); Group-V: Hydroethanolic extract of _Curcuma zedoaria_ (400 mg/kg body wt.). The extract was administered orally for 12 days. Two different dose levels of 200 and 400 mg/kg were used in this study. The hyperlipidemia was induced by single intraperitoneal injection of poloxamer (1 gm/kg body wt.) 48 h prior to blood collection. On 14th day, the blood was collected by retroorbital sinus puncture under light anesthesia. The blood was centrifuged at 3000 rpm for 10 min. The plasma was separated and was used for various biochemical estimations like total cholesterol, triglycerides, HDL cholesterol (Megalli et al 2005).

**Tissue homogenate preparation:**

On 14th day, the animals were sacrificed after blood collection, liver and heart were separated and sliced into pieces and homogenized in 10% KCl in cold conditions to give 10% homogenate. The homogenate was centrifuged at 3000 rpm for 20 min and the supernatant was used for protein estimation. Superoxide dismutase assay, catalase enzyme assay and lipid peroxidation assay were done and thiobarbituric acid reactive substances (TBARS) were estimated. Biochemical parameters like lipid profile in plasma such as total cholesterol, triglycerides, HDL cholesterol were estimated by using specific kits from ecoline diagnostic kits manufactured by Merck and Aldrich. LDL, VLDL, AI and HDL ratio were estimated by using the formula (Nanjian et al 2007). During _in vitro_ antioxidant studies, total protein by Bradford method (Ming et al 2009) and thiobarbituric acid reactive substances (TBARS) superoxide dismutase (SOD) and Catalase enzyme activity (CAT) were estimated.

**Statistical analysis**

The data collected from the results were subjected to one way ANOVA followed by student t-test using Graph Pad InStat statistical program.

**RESULTS AND DISCUSSION**

**Results**

The percentage yield of the extract was found to be 3.0805%. The extract of _Curcuma zedoaria_ was subjected to chemical tests as per the standard method for the identification of various constituents. _Curcuma zedoaria_ rhizome showed the presence of alkaloids. Total phenol content was found to be 34.45726±1.9 expressed as
mg/g equivalent of gallic acid. Total flavonol content was found to be 45.56667± 2.38 mg/g equivalent of quercetin. In vitro antioxidant activity, IC50 value for hydroethanolic extract was found to be 930±16.35 for DPPH method, >1000 µg/ml for nitric oxide method. Concentration required for reducing power was found to be 2.525±0.023 µg/ml. Total antioxidant capacity was found to be 230.2±1.32 which is expressed as mM equivalent of ascorbic acid (Table 3, 4). The effect of Curcuma zedoaria on lipid levels was determined by oral administration to rats for 12 consecutive days, after which hyperlipidemia was induced by injecting poloxamer 407 48 h prior to blood collection. It was found to be effective in reducing TC levels after 12 days of pretreatment with extract at a dose of 200 and 400 mg/kg body wt. reduced by 17.1% and 19.65% (Table 1). No significant changes were seen on LDL, VLDL and HDL cholesterol levels. All these levels observed were statistically significant (P<0.001) and indicated antihyperlipidemic activity of Curcuma zedoaria (Table 2).

Table 1. Effect of Curcuma zedoaria on plasma lipids in poloxamer 407 induced rats

<table>
<thead>
<tr>
<th>Group</th>
<th>TC (mg/dl)</th>
<th>TG (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
<th>VLDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>84.33±5.750</td>
<td>35.67±2.733</td>
<td>22.67±4.033</td>
<td>68.8±8.986</td>
<td>7.13±0.546</td>
</tr>
<tr>
<td>Poloxamer control</td>
<td>287.50±12.661***</td>
<td>567.67±23.662***</td>
<td>45.16±7.278***</td>
<td>355.87±13.42***</td>
<td>113.53±4.7***</td>
</tr>
<tr>
<td>Poloxamer + Atorvastatin (75 mg/kg)</td>
<td>119.16±6.306****</td>
<td>247.33±10.6****</td>
<td>54.83±5.279***</td>
<td>113.89±6.33***</td>
<td>49.467±2.12***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (200 mg/kg)</td>
<td>238.33±5.981***</td>
<td>565.17±21.99***</td>
<td>23.83±1.169***</td>
<td>328.03±6.649***</td>
<td>113.03±4.39***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (400 mg/kg)</td>
<td>231.0±5.518***</td>
<td>540.5±15.33***</td>
<td>26.0±0.894***</td>
<td>313.1±7.693***</td>
<td>108.1±3.067***</td>
</tr>
</tbody>
</table>

All values are expressed as a mean±SD (n=6); ***P<0.001, *P<0.01 as compared to untreated control; ###P<0.001, #P<0.01, as compared to poloxamer control

Table 2. Effect of Curcuma zedoaria on plasma lipids in atherogenic index and HDL ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>Atherogenic index</th>
<th>HDL ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>2.84±0.846</td>
<td>38.17±12.46</td>
</tr>
<tr>
<td>Poloxamer control</td>
<td>5.38±0.415***</td>
<td>18.67±1.335*</td>
</tr>
<tr>
<td>Poloxamer + Atorvastatin (75 mg/kg)</td>
<td>1.175±1.08***</td>
<td>85.66±7.944***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (200 mg/kg)</td>
<td>9.03±0.433***</td>
<td>11.08±0.556***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (400 mg/kg)</td>
<td>7.89±0.338***</td>
<td>12.69±0.532***</td>
</tr>
</tbody>
</table>

All values are expressed as mean±SD (n=6); ***P<0.001, *P<0.01, as compared to untreated control; ***P<0.001, *P<0.01, as compared to poloxamer control

Table 3. In vivo antioxidant parameters in liver homogenate

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (mg/ml)</th>
<th>LPO nmol/mg of protein</th>
<th>SOD (U/mg of protein)</th>
<th>CAT (µmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>0.79±0.002</td>
<td>90.71±1.98</td>
<td>63.43±0.811</td>
<td>25.52±0.653</td>
</tr>
<tr>
<td>Poloxamer control</td>
<td>0.312±0.14***</td>
<td>219.67±4.082***</td>
<td>19.99±1.572***</td>
<td>11.34±0.719***</td>
</tr>
<tr>
<td>Poloxamer + Atorvastatin (75 mg/kg)</td>
<td>0.432±0.037***</td>
<td>148.46±1.382***</td>
<td>54.65±1.250***</td>
<td>21.44±0.660***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (200 mg/kg)</td>
<td>0.289±0.023***</td>
<td>204.25±2.765***</td>
<td>25.26±1.059***</td>
<td>12.34±0.389***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (400 mg/kg)</td>
<td>0.315±0.047***</td>
<td>199.45±4.727***</td>
<td>28.68±0.996***</td>
<td>13.48±0.395***</td>
</tr>
</tbody>
</table>

All values are expressed as a mean±SD (n=6); ***P<0.001, *P<0.01 as compared to untreated control; ***P<0.001, *P<0.01, as compared to poloxamer control

Discussion
The hydroethanolic extract of Curcuma Zedoaria showed only the presence of alkaloids. No other compounds were present in this extract. This study showed that the extract had very less antioxidant activity by DPPH method compared
to that of standard ascorbic acid. It was found to possess reducing power more than that of the standard ascorbic acid. It did not exhibit nitric oxide scavenging activity. Total antioxidant capacity was found to be moderate.

The extract of *Curcuma zedoaria* was found to be effective in reducing total cholesterol level but no significant changes were seen on LDL, VLDL and HDL cholesterol levels. The extract of *Curcuma zedoaria* was unable to cause reduction in the atherogenic index. No significant reduction in TBARS level in both liver and heart homogenates were observed. The hydroethanolic extract elevated the SOD and CAT level but it was not very significant to show its antioxidant property. All these levels were statistically significant (p<0.001) and indicated antihyperlipidemic activity of *Curcuma zedoaria* which might be due to the effect of alkaloids.

### Table 4. *In vivo* antioxidant parameters in heart homogenate

<table>
<thead>
<tr>
<th>Group</th>
<th>Protein (mg/ml)</th>
<th>LPO nmol/mg of protein</th>
<th>SOD (UI/mg of protein)</th>
<th>CAT (μmol/min/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated control</td>
<td>1.06±0.019</td>
<td>28.97±1.896</td>
<td>16.209±0.327</td>
<td>9.530±0.279</td>
</tr>
<tr>
<td>Poloxamer control</td>
<td>0.517±0.005***</td>
<td>96.574±2.547***</td>
<td>7.758±0.4184***</td>
<td>4.234±0.185***</td>
</tr>
<tr>
<td>Poloxamer + Atorvastatin (75 mg/kg)</td>
<td>1.520±0.026*** ***</td>
<td>36.678±1.018*** ***</td>
<td>14.864±0.227*** ***</td>
<td>9.214±0.412*** ***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (200 mg/kg)</td>
<td>1.290±0.016*** ***</td>
<td>52.656±1.578*** ***</td>
<td>7.902±0.237*** ***</td>
<td>4.313±0.117*** ***</td>
</tr>
<tr>
<td>Poloxamer + Hydroethanolic extract (400 mg/kg)</td>
<td>0.896±0.021*** ***</td>
<td>57.921±1.783*** ***</td>
<td>8.432±0.217*** ***</td>
<td>4.895±0.121*** ***</td>
</tr>
</tbody>
</table>

All values are expressed as a mean±SD (n=6); ***P<0.01, *P<0.01 as compared to untreated control; ***P<0.001, *P<0.01, as compared to poloxamer control

### REFERENCES


http://www.pacificbulbsociety.org/pbswiki/files/Curcuma/Curcuma_zedoaria_Foliage_AD.jpg

****