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Original Research Article

## Evaluation of antidiabetic, anti-inflammatory and LDL oxidation inhibitory potential of *Alpinia galanga* and *Alpinia calcarata*-An *in vitro* study

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### ABSTRACT

*Alpinia galanga* and *Alpinia calcarata* are two important aromatic medicinal plants belonging to the family Zingiberaceae. In the present study, dried rhizomes of *A. galanga* and *A. calcarata* were extracted with water and then fractionated with different solvents. Among the various solvent fractions, ethyl acetate (EA) fraction of both plants showed high polyphenolic content with better antioxidant activity. This polyphenol rich EA fraction was also screened for its antidiabetic and anti-inflammatory potential using *in vitro* assays. Results showed that EA fractions of these two plants had significant antihyperglycemic activity by inhibiting carbohydrate digesting enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) and by inhibiting glycation reaction. Inhibitory potential of EA fractions of both plants against protein denaturation and xanthine oxidase validates their anti-inflammatory potential. Moreover, EA fractions also exhibited significant LDL oxidation inhibition. The observed antidiabetic and anti-inflammatory properties of *A. galanga* and *A. calcarata* could be due to the synergic effect of polyphenols such as gallic acid and ellagic acid as well as bioactive alkaloid, berberine.

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## 1. Introduction

Diabetes mellitus is one of the major causes of death in both the developed and developing countries. Even though it is a non-communicable disease, the number of diabetic patients increases day by day. Individuals affected by diabetes are prone to complications such as atherosclerosis, retinopathy, neuropathy, nephropathy and inflammatory conditions (Asgarpanah et al., 2011).  $\alpha$ -Glucosidase and  $\alpha$ -amylase are the key enzymes involved in the digestion of carbohydrates (Ali et al., 2006; Nickavar and Yousefian, 2009). By inhibiting these enzymes, the sudden increase of blood glucose level can be prevented (Kim et al., 2005). Therefore, such enzyme inhibitors can be useful in the treatment of type 2 diabetes (Schafer and Hogger, 2007). Additionally, free radicals have been shown to participate in glycation reaction, a reaction between aldehyde group of sugar and amino group of protein, resulting in the formation

of advanced glycated end products (AGES). The contribution of AGES to diabetes, aging and Alzheimer's disease has received a great deal of attention in recent years (Halliwell, 2001).

Individuals with diabetes have a higher risk for atherosclerotic cardiovascular diseases than non-diabetic patients. Oxidation of low-density lipoprotein (LDL) has been implicated as one of the major reasons for human atherosclerosis (Witztum and Steinberg, 1991). The dietary antioxidants and free radical scavengers are able to prevent LDL oxidation and AGES formation which can reduce the risk of atherogenesis and diabetes (Kinsella et al., 1993; Nakagawa et al., 2002). Consequently, extensive research is going on in this area, mainly focusing on the identification of natural extracts or organic compounds which can prevent the glycation reaction and oxidation of LDL (Prathapan et al., 2012).

Inflammatory conditions are also observed in diabetic

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patients. Carrageenan-induced mouse paw edema is frequently used to determine the anti-inflammatory activity of diverse bioactive compounds such as plant extracts and essential oils (Miguel, 2010; Das et al., 2012). There are certain difficulties when we use animal models for the experimental and pharmacological research, such as ethical issues and problems of handling and maintaining animals (Chandra et al., 2012). Thus, there is a need for much easier and efficient methods before further development of the animal models. Denaturation of tissue protein is a well-documented cause of inflammatory diseases. Any agent (natural/synthetic) that can prevent denaturation of proteins would be worthwhile for anti-inflammatory drug development (Chandra et al., 2012). Another main target in the treatment of inflammation and associated disorders includes an enzyme called xanthine oxidase (XO). There is an overwhelming acceptance that XO serum levels are increased in various pathological states like hepatitis, inflammation, ischemia-reperfusion, cancer, and aging (Berry and Hare, 2004). Thus, the selective inhibition of XO may result in broad-spectrum therapeutics for gout, cancer, inflammation and oxidative damage.

The medicine available for the treatment of diabetics such as acarbose, miglitol, nojirimycin and 1-deoxynojirimycin may cause serious side effects (Nickavar and Yousefian, 2009). Similarly, drugs which are widely used for the treatment of pain and inflammatory conditions, namely non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids, carry serious complications such as gastrointestinal problems, heart attack, stroke, high blood pressure and anemia (Meek et al., 2010; Sostres et al., 2013). The synthetic XO inhibitors such as allopurinol also reported to have many side effects such as hypersensitivity (Stevens-Johnsons) syndrome characterized by fever, skin rash, hepatitis, leukocytosis with eosinophilia and worsening renal function (Burke et al., 2006). The search for novel natural antidiabetic and anti-inflammatory agents with fewer side effects and multiple biological activities are essential for the management of diabetes and associated complications.

Indian system of medicine, Ayurveda, describes several medicinal plants with potent antidiabetic and anti-inflammatory activities. The advantages of the herbal medicines include better efficacy, less adverse effects, and comparatively low cost (Pathak and Das, 2013). However, several plants are not scientifically validated for their efficacy. *A. galanga* and *A. calcarata* are two important aromatic medicinal plants belonging to the family Zingiberaceae. They are widely used in cooking, especially in Indonesian and Thai cuisines and also as a medicine in rheumatoid arthritis and in inflammatory conditions. Even though some literature data are available (Nagashekhar and Shivaprasad, 2006), systematic studies and comparative evaluation of various biological activities and the chemical constituents in these two plants are still lacking. The present study deals

with the evaluation of antioxidant, antidiabetic, anti-inflammatory and LDL oxidation inhibition potential of *A. galanga* and *A. calcarata* along with the identification of the bioactive compounds present in the active fraction of these plants.

## 2. Experimental

### 2.1. Plant materials

The medicinal plants were collected from Kottayam district, Kerala, India and authenticated by Mrs. S. Komala Sanath (Former professor, Department of Botany, Guruvayoorapan College, Calicut, India). A voucher specimen of both *A. galanga* (MKU/NPC/004) and *A. calcarata* (MKU/NPC/005) were deposited in our lab for future reference.

### 2.2. Chemicals and reagents

All chemicals and reagents used were of standard analytical grades and were purchased from Sigma-Aldrich (St. Louis, MO, USA), Merck (Darmstadt, Germany) and Sisco Research Laboratories (Mumbai, India).

### 2.3. Extraction of plant material

Rhizomes of *A. galanga* and *A. calcarata* were dried (40-45 °C), powdered and extracted (500 g) with water (in ratio 1:10) at room temperature (27 ± 2 °C) under stirring for 3 h. The crude aqueous extracts of each plant were further fractionated into hexane, ethyl acetate and water fractions as per the method of Duan et al. (2006). Finally, each fraction was concentrated under reduced pressure and kept in an air tight container at 4 °C.

### 2.4. Estimation of Total Phenolic Content (TPC)

TPC of each extract was determined using Folin-Ciocalteu's reagent (FCR) method (Prathapan et al., 2011). Briefly, 100 µL of different extracts (three replicates), 500 µL of Folin-Ciocalteu's reagent and 0.4 mL sodium carbonate (20%) were added and incubated at ambient temperature (25-27 °C) for 90 min and the absorbance was measured at 760 nm using UV-Vis spectrophotometer (JASCO V-550, Japan). TPC of the extract was expressed as milligram of gallic acid equivalents/g plant extract (mg GAE/g).

### 2.5. Estimation of total flavonoid content (TFC)

TFC was determined by a colorimetric AlCl<sub>3</sub> method (Jia et al., 1999). Accordingly, about 100 µL of extract was added to the volumetric flask containing 3.9 mL of water, followed by addition of 300 µL of sodium nitrite (5.0% w/v) and the resulting mixture was kept for 6 min. To this system, 300 µL of 10% (w/v) AlCl<sub>3</sub> was

added to form a flavonoid-aluminium complex. After the incubation period, 2 mL of 4.3% (w/v) NaOH was added and the total volume was made up to 10 mL with distilled water and the absorbance was measured at 510 nm. TFC of the extract was expressed as milligram of quercetin equivalents/gram plant extract (mg QE/g).

#### 2.6. Estimation of Proanthocyanidin content (PaC)

PaC in the extracts was estimated by vanillin assay method (Sun et al., 1998). The 6.0 mL reaction system contained 1.0 mL extract, 2.5 mL vanillin in methanol (1.0%) and 2.5 mL of HCl in methanol (5.0 N). The reaction medium was incubated for 15 min at 30 °C and the corresponding absorbance was taken at 500 nm. PaC was expressed in terms of milligram of catechin equivalents per gram of extract (mg CE/g).

#### 2.7. Determination of DPPH radical scavenging activity

The antioxidant activity of extracts was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH method (Shimada et al., 1992). In this regard, the reaction mixture containing 2.8 mL of methanolic DPPH and 0.2 mL of extract was mixed well and incubated for 30 min at room temperature (25-29 °C). The absorbance was recorded at 517 nm.

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

ABTS assay was widely used to estimate the free radical quenching properties of hydrophilic and lipophilic compounds. To determine the radical scavenging activity of the prepared extracts using the ABTS assay, 980 µL of ABTS<sup>+</sup> reagent was mixed with 20 µL of the sample. Finally, the absorbance was taken after 6 min at 734 nm (Re et al., 1999).

#### 2.9. Determination of Total Antioxidant Activity (TAA)

TAA of the extracts was determined using 0.3 mL of sample mixed with 3 mL of a reagent solution consisting of 0.6 M H<sub>2</sub>SO<sub>4</sub>, 28 mM sodium phosphate and 4 mM ammonium molybdate. Reaction mixture was incubated at 95 °C for 30 min in water bath and the absorbance was taken at 695 nm (Nampoothiri et al., 2015).

#### 2.10. LC-MS/MS analysis

The combination of liquid chromatography-electrospray ionization mass spectroscopy technique (LC-ESI-MS/MS) was used for the quantification of phenolic compounds. The analysis was performed in a waters system (TQD detector with masslynx software). A C18 reverse phase column (Phenomenex (Torrance, USA) ODS-2.5 µm, 100 mm×4.6 mm) was used. Five micro liters of extracts (5 mg/mL in methanol) and standards (1 mg/mL) were loaded and injected by

autosampler and eluted through the column with a gradient mobile phase consisting of acetonitrile (A) and water (B) containing 0.2% formic acid as buffer, started from 5% A at 0 min, 20% at 15 min, 50% at 30 min and 100% at 30 min with a flow-rate of 0.6 mL/min in ESI-mode[M-H]<sup>-</sup>; capillary voltage 3.2V, cone voltage 30, source temperature 135 °C and a scan range 100-600 m/z.

#### 2.11. HPLC quantification of berberine

HPLC was used for the analysis of berberine. A C18 reverse phase column (Phenomenex (Torrance, USA) ODS-2.5 µm, 50 mm×4.6 mm) was used for the separation. Five micro liters of extracts (5 mg/mL in methanol) and standards (1 mg/mL) were loaded and injected manually and eluted through the column with an isocratic mobile phase system consisting of 1% formic acid in water (A) and acetonitrile (B) in the ratio of 99:1, with a flow-rate of 0.3 mL/min. The wavelength of the detector was set at 350 nm, the  $\lambda_{\max}$  of berberine.

#### 2.12. Validation of methods for LC-MS and HPLC analysis

LC-MS and HPLC analysis validated according to the standards of international Conference Harmonization (ICH). By comparing the retention time (RT) and mass spectra of the peaks with those of reference standards, the specificity of the method was determined. The linearity of the method was assessed by determining the detector responses to a series of solutions of reference standard of different concentration. Five analyses per concentration were conducted, and calibration plots were constructed. Limits of detection (LOD) and quantification (LOQ) of the methods were calculated using signal to noise ratio method. The precision of the method was validated in terms of repeatability and intermediate precision. The accuracy was determined by use of the standard addition technique. Standard addition experiments at each concentration were performed in triplicate and the accuracy was calculated as the % of the analyte recovered (Shabir, 2003; Nampoothiri et al., 2015).

#### 2.13. Evaluation of protein inhibition assay

The reaction mixture (5 mL) consisted of 0.2 mL of bovine serum albumin (1 mg/mL final concentration), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2.0 mL of varying concentrations of the sample. A similar volume of double-distilled water was used as control. The mixtures were then incubated at 37 ± 2 °C for 15 min and heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (Chandra et al., 2012).

#### 2.14. *In vitro* xanthine oxidase inhibition (XOI) activity



The assay mixture consisted of 1 mL of the test solution at different concentrations, 2.9 mL of phosphate buffer (pH 7.5) and 0.1 mL of enzyme solution. After pre-incubation at 25 °C for 15 min, the reaction was initiated by the addition of 2 mL of substrate solution (150 mmol xanthine in the same buffer). The assay mixture was incubated at 25 °C for 30 min. The reaction was then stopped by the addition of 1 mL of 1.0 N hydrochloric acid and the absorbance was measured at 290 nm (Prathapan et al., 2011).

## 2.15. Inhibition of key enzymes linked to diabetes

### 2.15.1. $\alpha$ -Amylase inhibition assay

Five hundred micro liters of extract and 500  $\mu$ L of sodium phosphate buffer (0.02 M) containing  $\alpha$ -amylase solution (0.5 mg/mL) were incubated at 25 °C for 10 min (Apostolidis et al., 2007). To this mixture, 500  $\mu$ L of a 1.0% starch solution was added to each tube at timed intervals and then incubated at 25 °C for 10 min. The reaction was stopped by addition of 1.0 mL of dinitrosalicylic acid as color reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled, diluted and absorbance was finally measured at 540 nm.

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

$\alpha$ -Glucosidase (20  $\mu$ L, 1.5 U/mL) premixed with 200  $\mu$ L of extracts at varying concentrations was made up in 50 mM phosphate buffer at pH 6.8 and incubated for 5 min at 37 °C (Shinde et al., 2008). 1 mM of *para*-nitrophenyl- $\alpha$ -D glucopyranoside (200  $\mu$ L) in 50 mM of phosphate buffer was added to initiate the reaction, and the mixture was further incubated at 37 °C for 20 min. The reaction was terminated by the addition of 500  $\mu$ L of 1.0 M Na<sub>2</sub>CO<sub>3</sub>, and the final volume was made up to 1500  $\mu$ L. The absorbance was recorded at 405 nm.

## 2.16. Antiglycation assay

About 500  $\mu$ L of albumin (1 mg/mL final concentration) was incubated with 400  $\mu$ L of glucose (500 mM) in the presence of 100  $\mu$ L of extract at different concentrations (Arom, 2005). The reaction was

allowed to proceed at 60 °C for 24 h and was stopped by adding 10  $\mu$ L of 100% TCA. Then, the mixture was kept at 4 °C for 10 min before subjection to centrifugation at 10000 rpm. The precipitate was redissolved in 500  $\mu$ L of an alkaline PBS (pH 10) solution and immediately quantified for relative amounts of glycated BSA based on fluorescence intensity at 370 nm (excitation) and 440 nm (emission).

## 2.17. Inhibition of human *in vitro* LDL oxidation

Oxidation of LDL leads to the production of malondialdehyde (MDA) which was measured by reaction with TBA (Kotamballi et al., 2002). LDL (50  $\mu$ g/mL) was incubated with different concentrations of the extract and the oxidation of LDL was initiated by the addition of 50  $\mu$ L of CuSO<sub>4</sub> (2 mM) at 37 °C for two hours. Final volume of the reaction mixture was made up to 1.5 mL with phosphate buffer (pH 7.4). After incubation, 500  $\mu$ L of the reaction mixture was mixed with 250  $\mu$ L of TBA (1.0% in 50 mM of NaOH) and TCA (0.28%). Samples were again incubated at 95 °C for 45 min. After cooling and centrifugation at 2000 rpm (10 min), fluorescence was recorded 553 nm emission.

## 2.18. 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 were calculated by Duncan's multiple range test using SPSS for windows, standard version 7.5.1, SPSS and the significance accepted at P $\leq$ 0.05.

## 3. Results and Discussion

### 3.1. Estimation of total phenolic, flavonoid and proanthocyanidin contents

Crude aqueous extracts of *A. galanga* and *A. calcarata* were fractionated into hexane (HE), ethyl acetate (EA) and water (WA) fractions. Among these, EA and WA fractions had high yield and these were taken for further analysis. The total phenolic (TPC), flavonoid (TFC) and proanthocyanidins (PaC) contents

**Table 1**

TPC, TFC and PaC in the ethyl acetate and water fractions of *A. galanga* and *A. calcarata*.

Plants	TPC <sup>a</sup> (mg GAE/g)	TFC <sup>b</sup> (mg QE/g)	PaC <sup>c</sup> (mg CE/g)
<i>A. galanga</i> EA	570.45 $\pm$ 1.5 <sup>a</sup>	270.37 $\pm$ 0.7	436.6 $\pm$ 6.36
<i>A. galanga</i> WA	40.5 $\pm$ 0.3	10.37 $\pm$ 0.05	330.6 $\pm$ 6.54
<i>A. calcarata</i> EA	630.66 $\pm$ 1.6	260.04 $\pm$ 0.70	379.0 $\pm$ 5.90
<i>A. calcarata</i> WA	30.20 $\pm$ 0.20	5 $\pm$ 0.04	310.0 $\pm$ 5.38

<sup>a</sup>TPC: Total phenolic content, <sup>b</sup>TFC: Total flavonoid content, <sup>c</sup>PaC: Proanthocyanidin content

<sup>d</sup>Each value represents mean  $\pm$  SD (standard deviation) from triplicate measurements (n=3) and the significance accepted at P $\leq$ 0.05.

**Table 2**

Evaluation of antioxidant activity of ethyl acetate and water fractions by different methods.

Plants/standards	DPPH	ABTS <sup>+</sup>	TAA
	( $\mu\text{g}/\text{mL}$ ) IC <sub>50</sub> values	( $\mu\text{g}/\text{mL}$ ) IC <sub>50</sub> values	(mg of ascorbic acid/g of extract)
<i>A. galanga</i> EA	4.8 ± 0.7 <sup>a</sup>	10.7 ± 0.3	650.7 ± 0.5
<i>A. galanga</i> WA	14.8 ± 0.8	19.7 ± 0.9	150.0 ± 0.1
<i>A. calcarata</i> EA	2.7 ± 0.4	10.5 ± 0.3	660.4 ± 0.5
<i>A. calcarata</i> WA	14.5 ± 0.8	18.5 ± 0.9	155.0 ± 0.1
Gallic acid	1.4 ± 0.3	0.8 ± 0.3	-
Ascorbic acid	7.3 ± 0.5	-	-
BHT	40.5 ± 3.0	-	-

<sup>a</sup>Each value represents mean ± SD (standard deviation) from triplicate measurements (n=3) and the significance accepted at P≤0.05.

of EA and WA fractions were evaluated (Table 1). The EA fractions of both plants had better phenolic, flavonoid and proanthocyanidin contents than the corresponding water fractions. Hence, further analysis was focused mainly on the EA fractions. Among the different fractions, EA fraction of *A. calcarata* had more phenolic and flavonoid composition than the other fractions even though the PaC was lower than EA fraction of *A. galanga*.

### 3.2. Evaluation of antioxidant activity

Antioxidant activities of the EA and WA fractions were evaluated by free radical scavenging assays (DPPH and ABTS methods) and by total antioxidant activity (Table 2). Results showed that the free radical scavenging activities of the EA fractions were found to be better than their crude aqueous extracts of *A. galanga* and *A. calcarata* of previous findings (Nampoothiri et al., 2015). The DPPH and ABTS radical scavenging abilities of EA fractions of *A. galanga* and *A. calcarata* were slightly less than that of gallic acid and even though

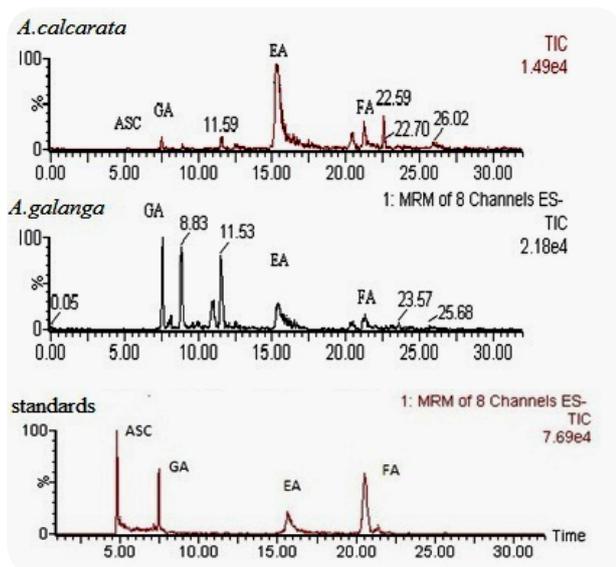
they were better than the synthetic antioxidant, BHT. TAA of two EA fractions also showed better activity than the corresponding WA fractions. The higher antioxidant activity of EA fractions were due to high amount TPC, TFC and PaC. In the overall antioxidant activity, EA fraction of *A. calcarata* had better antioxidant properties than *A. galanga* which was due to the high of TPC and TFC.

### 3.3. LC-MS/MS Analysis of EA fractions

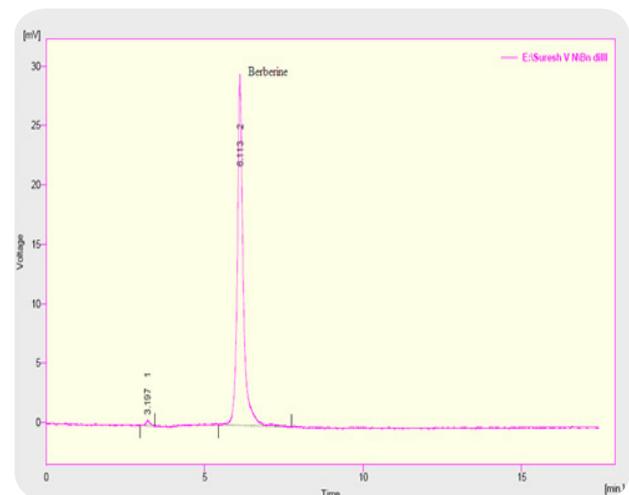
The combination of liquid chromatography-electrospray ionization mass spectroscopy technique (LC-ESI-MS/MS) was used for the quantification of phenolic compounds in the EA fractions of the two medicinal plants (Fig. 1). The estimated amounts of gallic acid and ellagic acid present in *A. galanga* and *A. calcarata* were 720 ± 2 and 935 ± 1, 365 ± 1 and 2605 ± 9 mg/kg respectively. The LC-MS/MS analysis revealed that gallic acid and ellagic acid were the major polyphenols present in the EA fraction of *A. galanga* and *A. calcarata* along with very small amounts of ferulic acid. Polyphenols are well-known natural antioxidants with several biological properties including anti-inflammatory and antidiabetic activities (Chandra et al., 2012).

### 3.4. Detection of berberine alkaloid in the EA fraction of *A. galanga* and *A. calcarata*

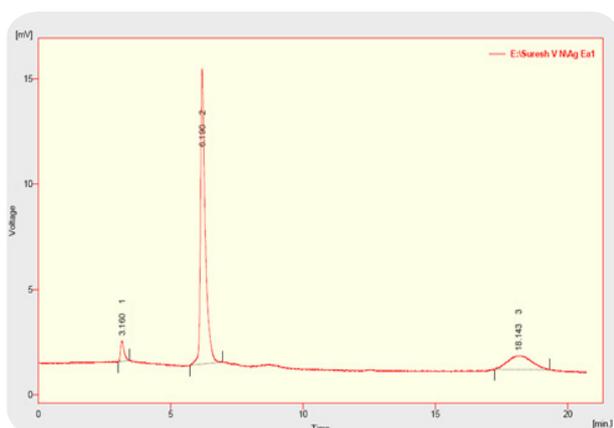
In accordance with Nampoothiri et al. (2017), the bioactive berberine alkaloid was also detected in *A. galanga* and *A. calcarata*. It is well mentioned that EA fraction contained the highest amount of berberine. The HPLC profiles of standard berberine, *A. galanga* and *A. calcarata* have been respectively presented in Fig. 2, Fig. 3 and Fig. 4 at 350 nm. The EA fraction of *A. calcarata* and *A. galanga* contained 1950 and 1680 mg/Kg of berberine, as well. The bioactive berberine alkaloid is well-known for its multiple therapeutic actions including antidiabetic, LDL cholesterol lowering



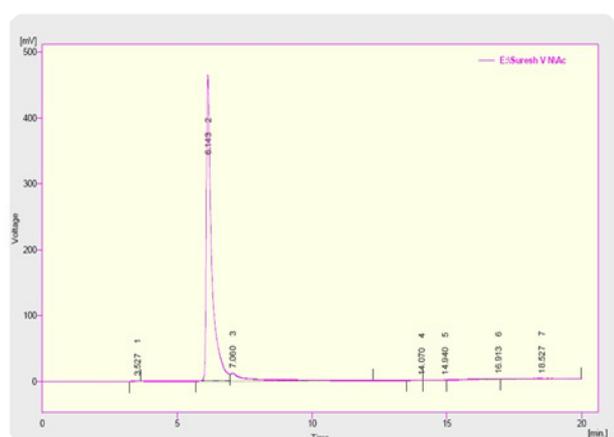
**Fig. 1.** LC-MS profile of ethyl acetate fractions of *A. galanga*, *A. calcarata* and standards; ASC: ascorbic acid, GA: gallic acid, EA: ellagic acid; FA: ferulic acid.



**Fig. 2.** HPLC profile of standard berberine.



**Fig. 3.** HPLC profile of *A. galanga* EA fraction.



**Fig. 4.** HPLC profile of *A. calcarata* EA fraction.

effect and anti-inflammatory activity (Yan-xia et al., 1995; Ivanovska and Philipov, 1996; Kong et al., 2004).

### 3.5. *In vitro* antidiabetic study

*In vitro* antidiabetic properties of the EA fractions of medicinal plants were evaluated by measuring their inhibition potential against the key enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase) linked to type 2 diabetes and the glycation reaction (Table 3). The EA fractions of *A. galanga* and *A. calcarata* significantly inhibited the carbohydrate digesting enzymes and showed comparable activity to that of the standard drug, acarbose having  $IC_{50}$  values 46.10 and 355.80  $\mu$ g/mL

**Table 3**

Inhibition of  $\alpha$ -amylase,  $\alpha$ -glucosidase, glycation, protein denaturation and xanthine oxidase by ethyl acetate fraction (EA) of *A. galanga* and *A. calcarata*.

Plant	Inhibition of $\alpha$ -amylase	Inhibition of $\alpha$ -glucosidase	antiglycation activity	Inhibition of protein denaturation	Inhibition of xanthine oxidase
<i>A. calcarata</i>	82.4 $\pm$ 0.7	74.8 $\pm$ 0.6	213.3 $\pm$ 1.1	44.9 $\pm$ 0.4	58.9 $\pm$ 0.6
<i>A. galanga</i>	83.9 $\pm$ 0.7	75.2 $\pm$ 0.6	252.4 $\pm$ 1.2	45.6 $\pm$ 0.4	68.4 $\pm$ 0.6
Positive control	355.80 <sup>a</sup>	46.10 <sup>a</sup>	30.50 <sup>b</sup>	618.52 $\pm$ 3.53 <sup>c</sup>	8.5 $\pm$ 0.6 <sup>d</sup>

The  $IC_{50}$  values are given in  $\mu$ g/mL and expressed as mean  $\pm$  SD (n=3).

<sup>a</sup>Acarbose ; <sup>b</sup>Ascorbic acid; <sup>c</sup>Diclofenac sodium; <sup>d</sup>Allopurinol

for  $\alpha$ -glucosidase and for  $\alpha$ -amylase respectively; which was in agreement with previous findings of Prathapan et al. (2012). An effective strategy for type 2 diabetes management is the strong inhibition of  $\alpha$ -glucosidase and mild inhibition of pancreatic  $\alpha$ -amylase (Krentz and Bailey, 2005), which was achieved by all of the four plant extracts. Glycation reaction plays an important role in the complication of diabetes. The present study reveals that EA fractions of *A. calcarata* and *A. galanga* showed significant antiglycation activity even though their activity slightly lower compared to the standard, ascorbic acid (30.50  $\mu$ g/mL). Among the two different EA fractions, EA fraction of *A. calcarata* exhibited better antidiabetic activity than *A. galanga*. A recent report shows that phyto bioactive compounds such as polyphenols, saponin, myrcelin, flavonoids, pectin, and glucosides rich extracts from the plants had significant antidiabetic activities (Gothai et al., 2016).

### 3.6. Inhibition of human LDL oxidation

The inhibition of human LDL oxidation by EA fraction of *A. calcarata*, *A. galanga* and standard compound, ascorbic acid resulted in  $IC_{50}$  ( $\mu$ g/mL) values as follows: 102.24  $\pm$  0.8, 97.67  $\pm$  0.80 and 24.5  $\pm$  0.94  $\mu$ g/mL, respectively. The obtained results showed that EA fractions of both plants significantly inhibited the LDL oxidation, which indicated that they can prevent the oxidation and also are able to reduce the infectious effects caused by the oxidation of LDL cholesterol. LDL oxidation is one of the major risk factors for atherosclerosis in humans and the persons suffering from diabetes have higher chance of cardiovascular complications mediated through LDL oxidation (Prathapan et al., 2012).

### 3.7. *In vitro* anti-inflammatory study

Anti-inflammatory potential of the EA fractions were evaluated in terms of their inhibitory potential against protein denaturation and xanthine oxidase (Table 3). EA fraction of *A. calcarata* had better inhibition of protein denaturation than diclofenac sodium, an anti-inflammatory reference drug ( $IC_{50}$ =618.52  $\pm$  3.53  $\mu$ g/mL). The xanthine oxidase inhibitory potential of the plant EA fractions and standards was as follows: Allopurinol >

*A. calcarata* > *A. galanga*. Even though the estimated  $IC_{50}$  values of plants were less than that of allopurinol ( $IC_{50}=8.5\pm 0.2\ \mu\text{g/mL}$ ), they showed significant inhibitory activity towards XO. As natural antioxidants, all these plant fractions were helpful in reducing the risk of inflammatory conditions by scavenging free radicals, inhibiting the XO and protein denaturation. The higher antioxidant, antidiabetic and anti-inflammatory properties of *A. galanga* and *A. calcarata* might be due to the synergic effect of polyphenols such as gallic acid and ellagic acid and also by the action of the berberine alkaloid. A report showed that plant antioxidants with significant free radical scavenging activity have multiple biological effects including antidiabetic, anti-inflammatory and inhibition towards LDL oxidation (Prathapan et al., 2012). The results of present study, thus authenticate the folklore information (Shivkanya et al., 2009) on the anti-inflammatory property of *A. galanga* and *A. calcarata*.

#### 4. Concluding remarks

The polyphenol rich EA fraction of *A. galanga* and *A. calcarata* screened for their *in vitro* biological activities such as antioxidant, anti-inflammatory, anti-hyperglycemic and inhibitory potential of LDL oxidation. The overall results revealed that the EA fraction of both plants contains significant amounts of phenolic compounds and exhibits potent antioxidant, anti-inflammatory, anti-hyperglycemic potential along with the capacity to inhibit LDL oxidation. Due to these beneficial biological activities, these plants could be used in the development of functional foods and nutraceuticals after detailed *in vivo* and clinical trials.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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