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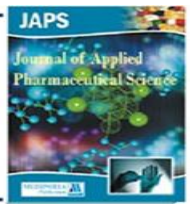

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Potential antidiabetic, hypolipidaemic and antioxidant effects of *Nymphaea pubescens* extract in alloxan induced diabetic rats

Shajeela .P .S, Kalpanadevi .V and Mohan .V .R

Shajeela . P .S
PG & Research Department of Botany, St. John's College, Palayamkottai 627002, Tamil Nadu, India

Kalpanadevi .V and Mohan .V .R
Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India.

For Correspondence
Mohan .V .R
Email: vrmohan_2005@yahoo.com

ABSTRACT

The present study was designed to investigate the possible antidiabetic, hypolipidaemic and antioxidant effects of ethanol extract of *Nymphaea pubescens* tuber. Diabetes was induced in Albino rats by administration of alloxan monohydrate (150 mg/kg, body weight i.p). The ethanol extract of *Nymphaea pubescens* tuber at a dose of 200mg/kg and 500mg/kg body weight were administered at single dose per day to diabetes induced rats for a period of 14 days. The effect of ethanol extract of *Nymphaea pubescens* tuber extract on blood glucose, plasma insulin, urea creatinine, glycosylated haemoglobin, serum lipid profile (total cholesterol (TC), triglyceride (TG), low density lipoprotein- cholesterol (LDL-C), very low density lipoprotein- cholesterol (VLDL-C) , high density lipoprotein- cholesterol (HDL-C)and phospholipids (PL)), serum protein, albumin, globulin, serum enzymes (Serum glutamate pyruvate transaminases (SGPT), serum glutamate oxaloacetate transaminases (SGOT) and alkaline phosphate (ALP)), lipoprotein peroxidation (LPO), blood reduced glutathione (GSH), oxidative glutathione (GSSG), GSH/GSSG ratio, erythrocytes glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione S-transferase (GST) were measured in the diabetic rats. The ethanol extracts of *Nymphaea pubescens* tuber elicited significant ($p<0.05$) reductions of blood glucose, lipid parameters except HDL-C, serum enzymes and significantly increased HDL-C and antioxidant. The extract also caused significant increase in plasma insulin ($p<0.05$) in the diabetic rats. In conclusion, ethanol extract of *Nymphaea pubescens* tuber offers promising antidiabetic and hypolipidaemic effects that may be mainly attributed to its potent antioxidant potential. Further studies will be needed in future in order to determine which one or more of its active constituents have the main antidiabetic and hypolidaemic effects.

Keywords: Diabetes, Alloxan, antioxidant, *Nymphaea pubescens*.

INTRODUCTION

Diabetes mellitus is probably the fastest growing metabolic disorder in the world and it is a major source of morbidity in developed countries. Once regarded as a single disease entity, diabetes is now regarded as a heterogenous group of diseases characterized by a state of chronic hyperglycemia, which causes a number of secondary complications like cardio-vascular, renal, neurological and ocular (Thornally *et al*., 1996). The incidence of diabetes mellitus is on the rise worldwide. Based on the World Health Organization (WHO) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 million or more by the year 2030 (Wild *et al*., 2004).

Diabetes mellitus is ranked seventh among the leading causes of death and is considered third when it's a total complications are taken into account (Trivedi *et al*., 2004). Plants are well known in traditional herbal medicine for their hypoglycaemic activities, and available literature indicate that there are more than 800 plant species showing hypoglycaemic activity (Rajagopal and Sasikala, 2008). There has been increasing demand for the use of plant products with antidiabetic activity due to low cost, easy availability and lesser side effects. Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycaemic agents. Flower extract of *Nymphaea nouchali* showed significant reduction of blood glucose in diabetic rats. Hydroalcoholic extract also showed a dose- dependent response possibly through β-cell stimulation, release of insulin receptors. *Nymphaea stellata* flower extract exhibited blood sugar lowering effect as well as antihyperlipidaemic effect on alloxan- induced diabetic rats. In view of above medicinal properties of *Nymphaea* spp., the present study was conducted to investigate the antidiabetic, antihyperlipidaemic and antioxidant activities of ethanol extract of *Nymphaea pubescens* tuber in alloxan induced diabetic rats.

MATERIALS AND METHODS

Plant material

Nymphaea pubescens tubers were freshly collected from the Injikuzhi, Agasthiarmalai Biosphere Reserve, Western Ghats, Tamil Nadu. The plant were identified and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu, India. A voucher specimen was deposited in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract for Phytochemical Screening and Antidiabetic Studies

The *Nymphaea pubescens* tubers were shade dried at room temperature and the dried tubers were powdered in a Wiley mill. Hundred grams of powdered *Nymphaea pubescens* tuber was packed in a Soxhlet apparatus and extracted with ethanol The extract were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures (Brinda *et al* ., 1981 ; Anonymous, 1990; Lala 1993).The ethanol extracts were concentrated in a rotary evaporator. The concentrated ethanol extract were used for antidiabetic studies.

Animals

Normal healthy male Wistar albino rats (180-240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12: 12 h).Rats were feed standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study (OECD 2002).The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50,100, and 2000 mg/kg body weight.

Induction of Experimental Diabetes

Rats were induced diabetes by the administration of simple intraperitoneal dose of alloxan monohydrate (150 mg/kg) (Nagappa et al., 2003).Two days after alloxan injection, rats screened for diabetes having glycosuria and hypoglycemia with blood glucose level of 200-260 mg/100 ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental design

In the investigation, a total of 25 rats (20 diabetic surviving rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

Group I: Normal, untreated rats.

Group II: Diabetic control rats

Group III: Diabetic rats given ethanol extract of *Nymphaea pubescens* tuber (200 mg/kg of body weight).

Group IV: Diabetic rats given ethanol extract of *Nymphaea pubescens* tuber (500 mg/kg of body weight).

Group V: Diabetic rats given standard drug glibenclamide (600µg/kg of body weight).

Biochemical analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000g for 10 minutes. Serum glucose was measured by the O-toluidine method (Sasaki *et al*., 1972). Insulin level was assayed by Enzyme Linked Immunosorbant Assay (ELISA) kit (Anderson *et al*., 1993). Urea estimation was carried out by the method of Varley (1976); serum creatinine was estimated by the method of Owen *et al* (1954). Glycosylated haemoglobin (HbA_1C) estimation was carried out by a modified colorimetric method of Karunanayake and Chandrasekharan (1985). Serum total cholesterol (TC) (Parekh and Jung, 1970), total triglycerides (TG) (Rice, 1970), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL- C) (Friedwald *et al*., 1972), high density lipoprotein cholesterol (HDL-C) (Warnick *et al*., 1985) and phospholipids (Takayama *et al*., 1977) were analyzed. Serum protein (Lowry, 1951) and serum albumins was determined by quantitative colorimetrically method by using bromocresol green. The total protein minus the albumin gives the globulin, serum glutamate pyruvate transaminase (SGPT) and serum glutamate

oxaloacetate transaminase (SGOT) was measured spectrophotometrically by utilizing the method of Reitman and Frankel (1957). Serum alkaline phosphatase (ALP) was measured by the method of King and Armstrong (1934). Catalase (CAT) (Bergmager, 1983), superoxide dismutase (SOD) (Madesh and Balasubramanian, 1998), lipid peroxidation (LPO) (Rehman, 1984), reduced glutathione (GSH) (Prins and Loos, 1969), Oxidised glutathione (Owen Joshua and Butterfield, 2010), glutathione reductase (GR) (Goldbery and Spooner, 1983), glutathione peroxidase (GPx) (Pagila and Valentine, 1967), and glutathione S-transferase (GST) (Habig *et al*., 1974) were analyzed in the normal, diabetic induced and drug treated rats.

Statistical analysis

The data were analyzed using student's t-test statistical methods. For the statistical tests a p values of less than 0.01 and 0.05 was taken as significant.

RESULTS AND DISCUSSION

The phytochemical screening of ethanol extract of *Nymphaea pubescens* tuber revealed the presence of alkaloids, flavonoids, glycosides,terpenoids, tannins, phenols, saponins and steroids. Acute toxicity study revealed the non-toxic nature of the ethanol extract *Nymphaea pubescens* tuber. Table 1 shows the levels of blood glucose, plasma insulin, urea, creatinine and glycosylated haemoglobin levels, while the plasma insulin level decreases significantly in alloxan induced diabetic rats (Group II) when compared with normal rats (Group I). Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Alloxan has a destructive effect on the beta cells of the pancreas (Jelodar *et al*., 2003; Prince and Menon, 2000). Alloxan causes a massive reduction in the insulin release by the destruction of beta cells of the islets of largerhans, thereby inducing hyperglycaemic (Grover *et al*., 2000). Insulin deficiency leads to various metabolic alterations in the animals viz, increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases (Begum and Shanmugasundaram, 1978; Shanmugasundaram *et al*., 1983). The results of the present study indicated that, *Nymphaea pubescens* tuber extract was found to reduce the glucose level and increase the plasma insulin significantly. The hypoglycaemic effect of *Nymphaea pubescens* tuber was found to be inducing insulin release from pancreatic cells of diabetic (Sharma and Garg, 2009). It is evident from this study that, there was an increase in insulin levels in diabetic rats treated with plant extract. A significant elevation in serum constituents, urea and creatinine were observed in alloxan induced diabetic rats (Group II) when compared to control rats. The results from the present study also indicated that, *Nymphaea pubescens* tuber extract can reduce the levels of serum urea and creatinine and confirms the protection of vital tissues (Kidney and liver) including the pancreas, thereby reducing the causation of diabetes in the experimental animals.

Alloxan induced diabetic rats showed significant increased ($p<0.05$) glycosylated haemoglobin (HbA_1C) level compared with normal rats. The ethanol extract of *Nymphaea pubescens* treated rats showed the decrease in the content of glycosylated haemoglobin. Glycosylated haemoglobin determinations are self monitoring of blood glucose therefore play important complementary roles for the management of diabetes mellitus (Thai *et al*., 1983).

The levels of serum protein, albumin and globulin of control, alloxan induced diabetic rats and drug treated rats were presented in Table 2. Reduction in serum protein, albumin and globulin were observed in alloxan induced diabetic rats (Group II) when compared to control (Group I). These values showed tendency to retrieve towards near normal values in ethanol extract of *Nymphaea pubescens* tuber administrated groups (Group III and IV) and glibenclamide (Group V) rats. These results were in accordance with the effect of *Wattakaka volubilis* and *Pterocarpus marsupium* in diabetic rats (Maruthupandian *et al*., 2010; Maruthupandian and Mohan 2011).

Table 2 summarized the effect of alloxan on the activity of the hepatic marker enzymes in serum. In the present study, SGPT, SGOT and ALP levels were increased significantly ($p<0.05$) in alloxan induced diabetic rats in respect to control group. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan (Stanely *et al*., 1999). In the present study, these elevated parameters in serum were come towards control level after treatment with *Nymphaea pubescens* tuber extract. The restorations of SGPT, SGOT and ALP to their respective normal levels after treatment with both glibenclamide and ethanol extract of *Nymphaea pubescens* tuber, further strengthen the antidiabegenic effect of these extract. Moreover, SGPT and SGOT levels also act as indicators of liver function and restoration of normal levels of these parameters indicate normal functioning of liver. Since the alloxan can also affect the liver by free radical mechanism.

The levels of serum lipid profiles, total cholesterol (TC), triglycerides (TG), LDL-C, VLDL-C, HDL-C and PL in control and diabetic rats were investigated (Table 3) .Alloxan induced diabetic rats showed significantly increased serum lipid profile except HDL-C, when compared with normal rats. The glibenclamide and ethanol extract of *Nymphaea pubescens* tuber treated rats showed a significant decrease in the content of lipid profile, when compared with diabetic induced rats. Similarly, HDL-C level decreased in alloxan induced diabetic rats when compared with normal rats. On administration of ethanol extract of *Nymphaea pubescens* tuber and glibenclamide to the diabetic rats, HDL-C level was found to be restored to normal. HDL helps to scavenge cholesterol from extra hepatic tissues (Brewer, 2004). Decreased HDL can contribute to the increased cholesterol levels. A greater increase of LDL may cause a greater decrease of HDL as there is a reciprocal relation between the concentration of LDL and HDL.

Phospholipids (PL) were increased in alloxan induced diabetic rats. Phospholipids are present in cell membrane and make up vast majority of the surface lipoprotein forming a lipid bilayer that acts as an interface with both polar plasm environment and

Table. 1: Effect of ethanol extract of *Nymphaea pubescens* on the serum glucose, insulin, urea, creatinine and glycosylated Hb level of normal, diabetic induced and drug treated adult albino rats.

Parameter	Insulin (MIu/ml)	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Glycosylated Hb (%)
Group I	20.4 ± 1.2	72.4 ± 3.8	13.34 ± 1.2	0.73 ± 0.1	4.8± 0.8
Group II	08.3 ± 0.8*	186.6 ± 9.7*	34.33 ± 2.8*	1.01 ± 0.4*	11.4±0.7*
Group III	14.2 ± 0.7[a]	92.5 ± 8.1[a]	16.24 ± 2.1	0.92 ± 0.7	8.9±0.7
Group IV	14.5 ± 0.9[a]	88.4 ± 6.1[a]	12.31 ± 2.3[a]	0.62 ± 0.5[a]	5.1±0.6[a]
Group V	16.54±0.5[a]	73.56±1.1[aa]	14.57±2.6	0.73±0.24	4.1±0.5[a]

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control, * P < 0.05 and comparisons were made between diabetic control to drug treated groups: a P<0.05; aa P<0.01 level

Table. 2: Effect of ethanol extract of *Nymphaea pubescens* on the protein, albumin, globulin, SGOT, SGPT and ALP level of normal, diabetic induced and drug treated adult albino rats.

Parameter	Protein (g/dl)	Albumin (g/dl)	Globulin(g/dl)	SGPT (u/l)	SGOT (u/l)	ALP (u/l)
Group I	6.71 ± 0.2	3.7 ± 0.1	3.0 ± 0.2	16.3 ± 5.8	24.70 ± 5.2	132.4 ± 4.9
Group II	3.74 ± 0.4	2.9 ± 0.2	1.1 ± 0.4	96.1 ± 3.4*	88.3 ± 7.3*	182.32 ± 6.4*
Group III	7.4 ± 0.2	4.7 ± 0.2	3.0 ± 0.4	26.3 ± 7.2	22.4 ± 4.1	111.52 ± 4.1
Group IV	6.8 ± 0.2	3.9 ± 0.4	3.0 ± 0.2	14.3 ± 5.8	19.2 ± 4.2	117.57 ± 8.9
Group V	7.1±0.3	4.0±0.2	3.1±0.1	17.5±2.1[a]	16.3±0.5[a]	103.2±5.8

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control and drug treated groups * P < 0.05 and comparisons were made between diabetic control to drug treated groups: a P<0.05

Table. 3: Effect of ethanol extract of *Nymphaea pubescens* on the TC, TG, LDL-C, VLDL-C, HDL-C and PL in the plasma of normal, diabetic induced rats.

Paramete	TC (mg/dl)	TG(mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	HDL -C (mg/dl)	PL (mg/dl)
Group I	98.67±2.98	72.33± 3.98	34.56 ± 1.98	14.46 ± 1.32	49.65 ± 2.11	155.83±5.66
Group II	172.81 ± 8.56*	236.66 ± 9.45*	105.93 ± 4.78*	47.33 ± 2.02*	19.55 ± 1.78*	208.23±8.89*
Group III	106.89 ±5.34[a]	142.34±4.65[a]	25.88±1.56[aa]	28.46±2.44[a]	52.55±2.87[aa]	162.75±7.34[a]
Group IV	89.23±2.98[a]	103.45±3.89[aa]	18.20±1.34[aa]	20.69±1.32[a]	50.34±2.54[a]	105.56±4.87[a]
Group V	104.45±6.98	98.56±3.45	39.43±1.67	19.65±1.22	46.34±1.98	160.56±5.78

Each value is SEM of 5 animals, Comparisons were made between normal control to diabetic control and drug treated groups * P < 0.05 and comparisons were made between diabetic control to drug treated groups: a P<0.05

Table. 4: Effect of ethanol extract of *Nymphaea pubescens* on the GSH, GSSG, GSH/GSSH, LPO, GR, GPx and GST activity of normal, diabetic induced and drug treated rats.

Parameter	Blood				Erythrocytes		
	GSH mol/mL	GSSG mol/mL	GSH/GSSG Ratio	LPO (nmol/mL)	GR nmol/min/mg protien	GPx nmol/min/mg protien	GST nmol/min/mg protien
Group I	1.56±0.08	0.08±0.004	19.5	0.89±0.067	4.845±0.87	74.67±11.23	62.45±6.56
Group II	1.02±0.04*	0.21±0.008*	4.8	2.78±0.037*	1.12±0.31*	46.67±10.32**	11.23±5.87**
Group III	0.97±0.03	0.09±0.003	10.7	2.56±0.067	1.09±0.34	83.45±11.23	35.54±7.88
Group IV	1.21±0.02	0.10±0.004	12.1	1.67±0.078	2.89±0.67	51.33±07.97	42.76±6.78
Group V	1.58±0.05	0.09±0.005	17.5	1.21±0.071	3.97±0.34	69.67±08.64	52.76±6.23

Each Value is SEM of 5 animals, Comparisons were made between normal control to diabetic control, * P < 0.05 ** P<0.01 and comparisons were made between diabetic control to drug treated groups: a P<0.05; aa P<0.01 level

non-polar lipoprotein or lipoprotein core (Cohn and Roth, 1996). Increased phospholipids levels in tissues were reported by Venkateswaran *et al* (2002); Pari and satheesh (2004) in Streptozotocin diabetic rats. Administration of ethanol extract of *Nymphaea pubescens* tuber and glibenclamide decreased the levels of phospholipids.

The increases in the levels of LPO due to the effects of diabetes are shown in Table 4. The results obtained showed that lipids of the diabetic rats are vulnerable to peroxidation due to the increased oxidative stress during diabetes. LPO plays an important role in aging, atherosclerosis and in a number of diabetic complications (Kesavulu *et al*., 2001; Matkovics *et al*., 1982). As diabetes and its complications are associated with free radical medicated cellular damage (Yu, 1994), herbal hypoglycaemic agents are administered to diabetic rats to assess their antioxidant potential. In the present study, *Nymphaea pubescens* extract not only have hypoglycemic activity but these compounds also significantly control the LPO levels in diabetic rats.

The level of GSH, GSSG, GSH/GSSH ratio in the blood and GR, GPx, GST in the erythrocytes of normal, diabetic induced and drug treated rats were studied. The highly significant reduction of the activity of scavenging mitochondrial enzymes are observed in alloxan induced rats. These adverse changes were reversed to near normal values in ethanol extract of *Nymphaea pubescens* (Table-4).

Mitochondria are the energy reservoir of the cells and the damage inflicted in mitochondria would ultimately result in the reduction of energy production and thereby leading to cell death (Sohal and Dubey, 1994). Subcellular membrane associated with thiol bearing enzymes, represents sensitive riskes for detoxification causing perpetuation of cellular function (Kyu and Byung, 1997). Reactive oxygen species can themselves reduce the activities of antioxidant defence mechanism. In the present study, ethanol extract of *Nymphaea pubescens* have enhanced mitochondrial enzymatic antioxidant activity and suppressed lipid peroxidation.

GSH is a major non- protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences (Uday *et al*., 1999). Decline in GSH content in the serum of diabetic induced rats, and its subsequent return towards near normally in plant extracts treated rats reveal the antioxidant effect of *Nymphaea pubescens*. Explanations of the possible mechanism underlying the antioxidant properties of this drug include the prevention of GSH depletion and destruction of free radicals (Valenzuela *et al*., 1985). These two factors are believed to attribute to the antioxidant properties of *Nymphaea pubescens*.

GPx is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. In hyperglycemia, glucose undergoes autooxidation and produces superoxide and it produces free radicals that inturn leads to lipid peroxidation in lipoproteins. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In the present study, decline in the activities of these enzymes in alloxan-induced diabetic animals and attainment of near normalcy in diabetic induced plant extracts treated groups indicate the oxidative stress elicited by alloxan had been nullified due to the effect of the extracts. This observation perfectly agrees with those of hypoglycemic and antioxidant activity of *Salacia oblonga* (Krishnakumar, 1999).

GSSG of blood was increased significantly in diabetic induced animals in comparison with control group. The ethanol extracts of *Nymphaea pubescens* treated rats decreased the levels of GSSG when compared with diabetic rats. The GSH / GSSG ratio in blood was also decreased significantly in diabetic induced animals, but in the plant extract treated group the decrease was less, in comparison with the control animals. Whenever GSH / GSSG ratio decreases, there is an adverse effect on several key enzymes of glycolysis (Gilbert, 1982). Increased amount of GSSG are transported out of cells to maintain the normal ratio (Eklow *et al*., 1981) but when accumulated inside the cell, GSSG creates oxidative stress, and various cellular components become vulnerable to damage by reactive oxygen species mainly membrane lipids, protein and DNA. GSH / GSSG ratio is maintained by enzymatic activities of GR and GPx. GR converts GSSG to GSH in the presence of NADPH, while GPx acts as an antioxidant. Glutathione –S-transferase (GST) activity was decreased significantly in the diabetic induced rats. The decrease in GR activity in diabetic induced animals may be responsible for the higher levels of GSSG. The present study indicates a reduction in the activity of GR, GPx, GST in alloxan induced rats. These results revealed the protective role of plant extract in decreasing lipid peroxidation and by normalizing antioxidant system.

In conclusion, ethanol extract of *Nymphaea pubescens* tuber offers a promising therapeutic value in prevention of diabetes. These effects could be mainly attributed to its antioxidant properties as shown by significant quenching impact on the extract of lipid peroxidation along with, enhancement of antioxidant defense systems in pancreatic tissue. The antioxidative property of *Nymphaea pubescens* extract certainly is due to its chemical constituents. Phytochemical investigations of *Nymphaea pubescens* have demonstrated the presence of flavonoids and phenolic compounds as main active ingredients having potent antioxidant activities. Further studies will be needed in future to determine the main active ingredient having the beneficial antidiabetic, hypolipidaemic and antioxidant effects.

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