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Original Article

In vitro Hypoglycemic and Antioxidant Activities of Litsea cubeba (Lour.) Pers. Fruits, Traditionally Used to Cure Diabetes in Darjeeling Hills (India)

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ABSTRACT

Introduction: Fruits of *Litsea cubeba* (Lour.) Pers. has been reported to be used traditionally in the treatment of diabetes in Darjeeling Himalayan region of India; though the hypoglycemic potential of the fruit has not been assessed till now, and the active constituents are yet to be discovered. Therefore, there is a necessity for the appraisal and characterization of the hypoglycemic properties of the fruits. **Methods:** Fresh fruits were collected and sequentially extracted with solvents of gradient polarity. *In vitro* antidiabetic activity was performed by α-amylase and α-glucosidase inhibitory assays. Free radical scavenging activity was performed by DPPH, ABTS, TPTZ (FRAP assay), NO and OH radical scavenging assays. To identify the bioactive components, GC-MS analysis was also performed. **Result:** Phytochemical screening of secondary metabolites in different solvent extracts showed the presence of phenols, flavonoids, alkaloids, cardiac glycosides, tannins, saponins, and anthocyanins. Methanolic extract exhibited highest antidiabetic potential with IC_{50} values of 514.9 µg/ml and 1435.7 µg/ml in α-amylase and α-glucosidase inhibition assay respectively followed by ethanol extract. Significant free radical scavenging activities were also found in the alcohol extracts. GC-MS analysis revealed the presence of principle compounds like oleic acid, morin, apigenin etc. which might be responsible for hypoglycemic activity. **Conclusion:** Here we report the appraisal of traditional usage of *L. cubeba* (Lour.) Pers. fruits based on *in vitro* antidiabetic and antioxidant assays along with GC-MS characterization of potent molecules. Our study confirms the traditional knowledge of the people of Darjeeling Hills regarding the use of the fruit of this plant in curing diabetes.

Key words: *Litsea cubeba* (Lour.) Pers, Antidiabetic, Antioxidative, GC-MS analysis.

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INTRODUCTION

Diabetes is one of the most dreadful diseases in terms of high mortality rate all over the world. High fasting and postprandial blood sugar level (hyperglycemia) is the main diagnostic feature of Diabetes that may be either due to insulin deficiency (Type-1) or impaired insulin action (Type-2) inside the body.[1] Apart from hyperglycemia, a couple of severe macro and micro-vascular complications are usually associated with diabetic patients affecting multiple organs at a time. Macro-vascular complications include coronary heart disease and stroke that are responsible for high rate of diabetic mortality and morbidity compared to the microvascular diseases (retinopathy, neuropathy, and nephropathy).[2]

Development and progression of diabetic symptoms are closely associated with increased oxidative stress within the body. It is already proved that oxidative stress plays a key role in developing vascular diseases by endothelial dysfunction.[3] An elevated level of blood glucose triggers rapid production of highly reactive oxygen and nitrogen species (ROS and RNS) which in turn destabilizes cellular antioxidant defense mechanism. Activation of transcription factors and protein kinases, excessive production of advanced glycation end products may act together in the overall oxidative stress development.[4] Superoxide dismutase, catalase, glutathione peroxidase etc. are strongly affected by the excess production of free radicals which ultimately disrupt body immune system. Also collapsed glycemic control may result in inflammatory reactions that are responsible for visceral fat deposition, hyperlipidemia, hypertension and cardiovascular diseases.[5] However, the precise mechanism behind the development of oxidative stress during diabetes is yet to be fully deciphered.

Presently, the treatment of diabetes relies mostly on the synthetic medicines and insulin therapy which could only relieve the preliminary sufferings but can never be the ultimate solution to the problem. Also, it is rather impossible to

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reduce the distress of the multiple adverse side effects *viz.* kidney and liver diseases.[6] Use of herbal medicine in curing diseases has been a long-term practice and already manifested to be a cost-effective and harmless reliever. The proposed mechanism of action of herbal therapy includes regulation of metabolism, prevention of β cell destruction, modulation of glucose absorption, enhancement of insulin secretion etc.[7] Many plants (*Momordica charantia* L., *Azadirachta indica* A. Juss., *Swertia chirayita* (Roxb. ex Flem.), *Trigonella foenum-graecum* L. etc.) have already been recognized as hypoglycemic with their spectral disease-curing properties.[8]

The genus *Litsea* Lam. of the family Lauraceae comprises of 151 species as per the record of accepted names enlisted in 'The Plant List' database.[9] *Litsea cubeba* (Lour.) Pers. is a wild evergreen tree geographically distributed from the eastern Himalayas to South-East Asia traversing the countries of India, China, Taiwan, and Malaysia.[10] Approximately, 20 species of this genus has been in use in China for long in several indigenous herbal formulations for the treatment of gastrointestinal diseases, asthma, arthritis, pain management etc.[11] Fruits are even used as spicy condiments in the indigenous cuisine of Taiwan and popularly known as *may chang* (mountain pepper) in Mandarin.[12] The fruits are also used as carminative and in the preservation of fish in Indonesia.[13] This species, in particular, is priced for its essential oils having antifungal, antibacterial, insecticidal and anticancer activities.[14-19] The major components in the oils extracted from different parts of the plants were ascertained to be citral, β-phellandrene, and β-terpinene. The role of the essential oil in dendritic cell inhibition was reported that could be attributed to its potent immunosuppressive properties.[20] The oil is also used as an active ingredient of aromatherapy and personal care products.[21] Citral (neral and geranial) which is approximately 80% of the extracted essential oils are reported to be potent free radical scavengers and thus could act as a source of natural antioxidants.[22]

In India, the fruits and seeds are edible and usually consumed either raw or cooked in the form of pickle and chutney by many ethnic groups.[23-24] Use of *L. cubeba* as spice is also a common practice in different parts of India.[25] In Arunachal Pradesh, the fruits are believed to confer beneficial and protective properties in heart and stomach disorders.[26] Flowers and fruits are used in the treatment of a sore throat by Chiru tribal community in Manipur.[27] This plant is also reported to be in use by the natives of the Darjeeling and Sikkim Himalayan region for the treatment of diabetes. Ethnic communities in this region often consume one or two raw fruits as masticatory and also as pickle to prevent and check hyperglycemia.[28] Though the fruits have been used traditionally to control diabetes, but till now there are no such scientific reports claiming for the validation of the antidiabetic property of *L. cubeba* from this region. The antidiabetic property of the bark of this plant has been reported once previously from Western Java (Indonesia); pointing towards the antidiabetic virtue of this plant.[29] Also, several other species of *Litsea* have been reported to possess antidiabetic properties. Lu *et al*. reported the potent role of the flavonoid contents extracted from the leaves of *L. coreana* in lowering blood sugar level, hyperlipidemia and inflammation in experimentally induced type-2 diabetic rats.[30] Similarly, the ethanolic and methanolic extracts of *L. japonica* and *L. glutinosa* respectively also exhibited antidiabetic activity.[31-32]

The article thus aims to validate the hypoglycemic property of different solvent extracts in correlation with the free radical scavenging properties of *L. cubeba* fruits collected from Darjeeling Hills and also the preliminary characterization of active constituents through GC-MS. Further works in this regard is going on for the characterization of novel antidiabetic phytoconstituent(s) that might contribute to alleviating human suffering of type-2 diabetes.

MATERIALS AND METHODS

Plant material

Plant material (fresh fruits of *L. cubeba* (Lour.) Pers.) were collected from different localities of Chatakpur Forest (latitude 26º 96´N and longitude 88º 30´ E) and Dow Hill Forest (latitude 26º 89´N and longitude 88º 27´E) of Kurseong Sub-Division of Darjeeling District during the months of May to July 2016. A herbarium specimen (voucher specimen no. UGB/BOT/RC/01) was deposited at NBU-Herbarium (acronym: NBU), Department Of Botany, North Bengal University, Siliguri-734430, West Bengal for authentication (accession no. 10046). The collected fruits were shade-dried for about 15 days under the well-aerated condition at 25ºC. The dried fruits were then thoroughly washed under tap water, dried and ground to make a fine powder using a mechanical grinder. The powdered plant material was stored at room temperature for further use.

Preparation of plant extracts and estimation of extractive values

The powdered plant material was sequentially extracted in a Soxhlet apparatus using different solvents with their increasing polarity *viz.* hexane, acetone, methanol, ethanol and distilled water according to the method previously described.[33] 100 g of powdered material was extracted using 250 ml of hexane for 24-48 h and the extract was filtered using Whatman no.1 filter paper. The residue was air-dried and sequentially extracted using other solvents. The pooled solvents following the extraction procedure were concentrated using rotary evaporator under reduced pressure and percentage yields of each solvent were determined. The concentrated extracts were stored at 4ºC for future use.

Phytochemical screening of extracts

Qualitative screening for the presence of phytochemicals was carried out from the concentrated plant extracts following standard protocols.[34] Presence and absence of quinones, tannins, saponins, anthocyanins, carotenes, phlobatanins, alkaloids, flavonoids, phenols, cardiac glycosides and terpenoids were determined.

In vitro antidiabetic activity

α-amylase inhibitory assay

The assay of α-amylase inhibition was performed by quantifying the amount of reducing sugar liberated under assay conditions. A modified 3,5- dinitrosalicylic acid (DNS) method was adopted to estimate the maltose equivalent.[35] 1 ml of the plant extracts were pre-incubated with 1 Unit/ml α-amylase enzyme from porcine pancreas, Type VI-B (SIGMA) for 30 min and thereafter 1 ml(1% w/v) starch solution was added. The mixture was further incubated at 37°C for 10 min. Then the reaction was stopped by adding 1 ml DNS reagent and the contents were heated in a boiling water bath for 5 min. The absorbance was finally measured at 540 nm and acarbose was used as positive control.

α-glucosidase inhibitory assay

The α-glucosidase inhibitory assay was assessed following standard method with minor modifications.[36] The reaction mixture contained 1 ml of 2.5 mM 4-nitrophenyl α-D-glucopyranoside (PNP-G), 1.5 ml of phosphate buffer (pH 7.0) and 0.5 ml of plant extract at different concentrations. The reaction was initiated by the addition of 0.2 ml of 0.28 U/ml of the α-glucosidase enzyme from *Saccharomyces cerevisiae* (SIGMA) and subsequently incubated at 37°C for 30 min. The reaction was terminated by the addition of 1 ml of 0.2 M sodium carbonate solution. The enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released in the reaction mixture at 400 nm. Acarbose was used as positive control.

In vitro antioxidant assays

DPPH radical scavenging assay

Different concentrations of plant extracts (0.1 ml) were mixed with 2.9 ml of the methanolic solution of 0.1 mM DPPH (2,2-Diphenyl-1-picryl-hydrazyl).[37] The mixture was kept in the dark at room temperature for 30 min and absorbance was measured at 517 nm against a blank. Ascorbic acid was used as the standard.

ABTS radical scavenging assay

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) scavenging assay was carried out as per the standard protocol.[38] ABTS radical cation ($ABTS^{+}$) was produced by the reaction of a 7 mM ABTS solution with 2.45 mM of potassium persulphate. The $ABTS^{+}$ solution was then diluted with ethanol to an absorbance of 0.7 ± 0.05 at 734 nm and stored in the dark at room temperature for 12 h before use. After addition of 25 µl of plant extracts to 2 ml of diluted $ABTS^{+}$ solution, absorbance was measured at 734 nm after 6 min. Ascorbic acid was used as the standard.

FRAP assay

The FRAP assay is based on the reduction of Fe^{+3}-TPTZ to the colored form of Fe^{+2}-TPTZ.[39] The fresh FRAP reagent was prepared by dissolving 50 ml of 10 mM 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) and 50 ml of 50 mM $FeCl_3.6H_2O$ in 500 ml of acetate buffer (300 mM; pH 3.6). Subsequently, 75 µl of plant extracts (1mg/ml) was mixed with 2 ml of FRAP reagent and absorbance was measured at 593 nm in a spectrophotometer after 12 min. FRAP activity was finally calculated from the standard curve of $FeSO_4$ and expressed as mM equivalent of the Fe^{+2} ion. Ascorbic acid was used as the standard.

Nitric oxide scavenging assay

Nitric oxide scavenging activity was determined through Griess Illosvoy reaction.[40] Different concentrations of plant extracts were mixed with 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer (0.5M, pH 7.4) in a final volume of 3 ml. After incubation for 60 min at 37°C, Griess reagent [N-(1-Naphthyl) ethylenediamine (0.1%) and sulphanilic acid (1%) in H_3PO_4 (5%)] was added. Generation of pink chromophore was finally measured spectrophotometrically at 540 nm. Ascorbic acid was used as the standard.

Hydroxyl radical scavenging assay

The reaction mixture was prepared with 1.5 ml of plant extract of different concentrations, 60 µl of 1 mM $FeCl_3$, 90 µl of 1mM 1,10-phenanthroline, 2.4 ml of phosphate buffer (0.2 M; pH 7.8) and 150 µl of 0.17 M H_2O_2 and subsequently homogenised in a vortex.[41] After that, it was incubated at room temperature for 5 min and the absorbance was measured at 560 nm against the blank.

Calculation of IC_{25} and IC_{50} values

The IC_{25} and IC_{50} values of the *in vitro* antidiabetic and antioxidant activities of the extracts were calculated from the individual plots showing the percentage inhibition at different concentrations. The calculation was done following global curve fitting using nonlinear regression against four-parameter logistic function in SigmaPlot v14.0 according to the following formula:

$$Y\ (IC_{25}\text{ or }IC_{50}\text{ value}) = [(I_{min} - I_{max}) / 1 + (X / IC_{25\text{ or }50})^{H}] + I_{max}$$

I_{min} is the minimum percent inhibition, I_{max} is the maximum percent inhibition, H is the hill slope.

Quantification of antioxidative biochemicals

Total phenol content (TPC)

TPC was determined following the Folin-Ciocalteau method with minor modifications.[42] 200 µl of plant extracts (1mg/ml) was mixed with 800 µl of freshly prepared Folin-Ciocalteau reagent and 2 ml of 7.5% sodium carbonate. The final volume was made up to 7 ml of distilled water and allowed to stand in dark for about 2 h. The absorbance was measured at 765 nm. Gallic acid was used as standard and the TPC contents were expressed as mg of gallic acid (GA) / g dried extract.

Total flavonoid content (TFC)

TFC was determined using a modified aluminum chloride method.[43] 500 µl of plant extracts (1mg/ml) was added with 500 µl 5% $NaNO_2$ and incubated at room temperature for 5 min. Subsequently, 500 µl of 10% $AlCl_3$ was added to it and again incubated for 5 min. After the incubation period, 1 ml of 1 mM NaOH was added to the reaction mixture. The absorbance was finally recorded at 510 nm and the TFC contents were expressed as mg of quercetin (QE) / g dried extract.

GC-MS analysis

GC-MS analyses of the methanolic and ethanolic extracts were performed using JEOL GCMATE II to identify the bioactive constituents. For GC-MS detection, an electron impact ionization system was implemented with ionization energy of 70 eV. The helium gas was used as a carrier with a flow rate maintained at 1 ml/min and 2 µl injection volume was used with the injector temperature set at 250°C. The initial temperature of the oven was maintained at 100 °C for 5 min after that increased to 220°C at the rate of 4°C/min and maintained further for 20 min. The mass spectral scan was performed at 0.5 s interval and range of scan was from 50 to 400 m/z. The spectrums obtained through GC-MS were finally compared with the MS data library of National Institute of Standards and Technology (NIST) for the identification of bioactive compounds.

Statistical analysis

All the tests were performed in triplicate and the data were presented as mean ± S.D. Sigma Plot v14.0 was used for plotting the graphs and calculation of IC_{25} and IC_{50} values of the scavenging assays. Principal Component analysis (PCA) was performed to evaluate the relationships of all the variables, using SPSS 21.

RESULTS

Phytochemical screening of the extracts and extractive values

The presence and/or absence of different phytochemicals were presented in Table 1. It was observed that phenols, flavonoids and saponins were present in all the extracts. Only methanol extract showed the presence of phlobatanins. Alkaloids and cardiac glycosides were present in all the extracts except hexane. Quinones were present in methanol and acetone extracts and terpenoids were found in acetone and hexane extracts respectively. Both methanol and ethanol extracts showed positive result for tannins. Water, ethanol and methanol showed positive result for anthocyanins. Presence of carotenes was restricted among methanol, acetone and hexane extracts. The percentage extractive values were 1.0 ± 0.89(hexane), 5.7 ± 0.55(acetone), 8.1 ± 0.87(methanol), 8.0 ± 1.55 (ethanol) and 7.3 ± 1.17(water).

In vitro antidiabetic activity

α-amylase inhibitory assay

The enzyme inhibitory activity was increased according to the concentration of both the methanol and ethanol extracts, followed by water

Table 1: Screening of secondary metabolites in different solvent extracts of the fruit powder of *L. cubeba*.

Secondary metabolites	Hexane	Acetone	Methanol	Ethanol	Water
Phenols	+	++	+++	++	++
Flavonoids	+	+	+++	++	++
Alkaloids	–	+	++	++	++
Quinones	–	+	+	–	–
Terpenoids	++	+	–	–	–
Cardiac glycosides / Steroids	–	+	+	++	++
Tannins	–	–	++	+	–
Saponins	+	++	++	++	+
Anthocyanins	–	–	+	++	++
Carotenes	++	++	++	–	–
Phlobatannins	–	–	+	–	–

Here, the signs indicate '+++' = high presence, '++' = moderate presence, '+' = low presence, '–' = absent.

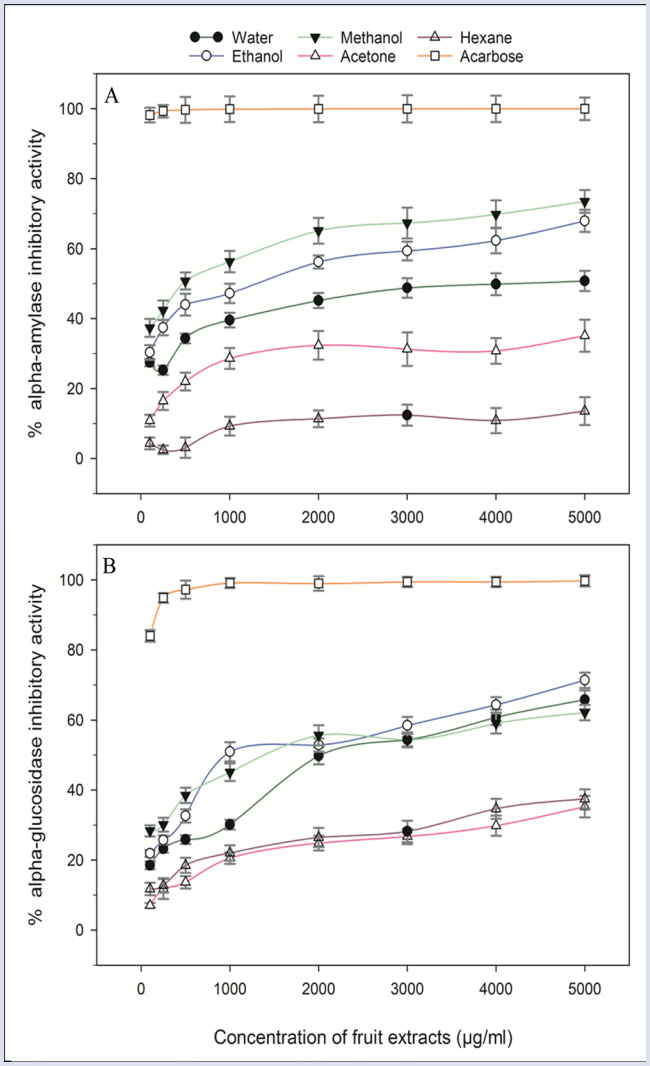

Figure 1: *In vitro* antidiabetic activities of the fruit extracts of *L. cubeba*. (A) α-amylase inhibitory activity. (B) α-glucosidase inhibitory activity. Here, the values represent the average of triplicate trials and the error bars are the standard deviation values.

extract (Figure 1A). The IC_{25} and IC_{50} value of the inhibitory activity of all the extracts was also listed in Table 2. The methanol extract showed the lowest IC_{50} value of 514.9 µg/ml followed by ethanol extract value of 1111.1 µg/ml. However, the remaining extracts (water, acetone and hexane) did not show any significant inhibitory activity as their IC_{50} values could not be determined from the range of concentrations tested. The IC_{25} and IC_{50} value of the standard drug acarbose could not be determined as the values were very low to be calculated, because it exhibited high activity even at very low concentrations.

α-glucosidase inhibitory assay

Inhibitory activity of the plant extracts was observed to increase with the concentration of both the ethanol and methanol extracts, followed by water extract (Figure 1B). The IC_{25} and IC_{50} values of the extracts are recorded in Table 2. Here the lowest IC_{50} value was recorded for ethanol extract (1426.5 µg/ml), closely followed by methanol extract (1435.7 µg/ml). However, hexane and acetone extracts failed to exhibit any significant inhibitory activity as their IC_{50} values could not be determined. The IC_{50} value of acarbose was determined to be 18.05 µg/ml, whereas the IC_{25} was even lower which could not be determined.

In vitro antioxidant assays

DPPH radical scavenging assay

The scavenging activity of different extracts was presented in Figure 2A. Ascorbic acid exhibited maximum scavenging activity followed by methanol, ethanol, water and acetone extracts, in a decreasing manner. No significant activity was observed in case of hexane extracts, as the IC_{50} value could not be determined. Concentration of extracts and standards in terms of their IC_{50} values were in the following order: Ascorbic acid > methanol extract > ethanol extract > water extract > acetone extract as shown in the Table 2.

ABTS radical scavenging assay

ABTS radical scavenging of different extracts was presented in Figure 2B. Significant scavenging activity was observed in methanol extracts in the concentration between 100-400 µg/ml. The IC_{50} values were in the following order: Ascorbic acid > methanol extract > ethanol extract > acetone extract > water extract, indicating their corresponding scavenging properties (Table 2). However, no observable activity was found in hexane extracts.

Table 2: IC_{25} **and** IC_{50} **values of *in vitro* antidiabetic and antioxidant activities of the different solvent extracts of fruit powder of *L. cubeba*.**

Parameters	IC_{25} and IC_{50} values	Hexane	Acetone	Methanol	Ethanol	Water	Acarbose / Ascorbic acid
α-amylase	IC_{25}	ND	653.9	ND	23.3	ND	ND
	IC_{50}	ND	ND	514.9	1111.1	4235.4	ND
α- -glucosidase	IC_{25}	1611.8	2016.1	ND	182.1	519.7	ND
	IC_{50}	ND	ND	1435.7	1426.5	2289.5	18.05
DPPH	IC_{25}	280.4	120.8	78.2	72.3	105.4	20.4
	IC_{50}	ND	823.6	308.7	330.5	577.4	69.3
ABTS	IC_{25}	177.8	56.9	32.8	79.8	104.6	ND
	IC_{50}	ND	307.2	121.9	243.9	732.4	32.1
NO	IC_{25}	ND	922.4	179.9	260.8	386.6	54.2
	IC_{50}	ND	ND	596.3	1078.6	ND	180.1
OH	IC_{25}	501.5	314.7	74.9	62.2	174.8	51.5
	IC_{50}	ND	ND	337.8	287.7	779.9	146.4

Here, 'ND' indicates that the IC values do not fall within the range of the concentrations of plant extracts and therefore could not be determined using SigmaPlot and acarbose / ascorbic acid used as standard.Acarbose is used as a standard for in vitro antidiabetic assays and Ascorbic acid is used a standard for in vitro antioxidant activity assays.

Table 3: Solvent extractive values, FRAP activity, total phenol and total flavonoid content of the different solvent extracts of fruit powder of *L. cubeba*.

Solvent extracts	Solvent Extractive Values (in %)	FRAP activity (mM Fe^{2+} equiv.)	Total Phenol Content (mg gallic acid equiv./g)	Total Flavonoid Content (mg quercetin equiv./g)
Hexane	1.0 ± 0.89	0.29 ± 0.017	1.37± 0.4	25.89± 0.92
Acetone	5.7 ± 0.55	0.16 ± 0.009	117.41± 5.09	51.35 ± 6.19
Methanol	8.1 ± 0.87	0.47 ± 0.023	182.43± 1.23	189.36 ± 5.81
Ethanol	8.0 ± 1.55	0.44 ± 0.018	120.53± 2.79	145.64± 2.26
Water	7.3 ± 1.17	0.24 ± 0.007	105.33 ± 2.01	111.6± 2.36

Here, the values are the average of triplicate trials with their standard deviation.

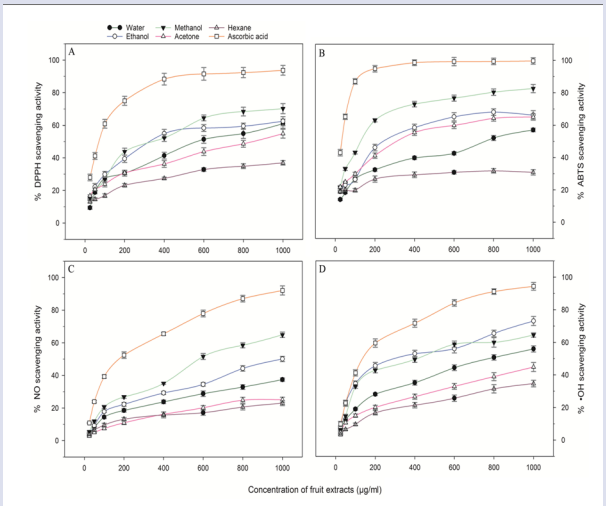

Figure 2: *In vitro* antioxidant activities of the fruit extracts of *L. cubeba*. (A) DPPH free radical scavenging. (B) ABTS radical scavenging. (C) NO radical scavenging. (D) OH radical scavenging. Here, the values are the average of triplicate trials and the error bars are the standard deviation values.

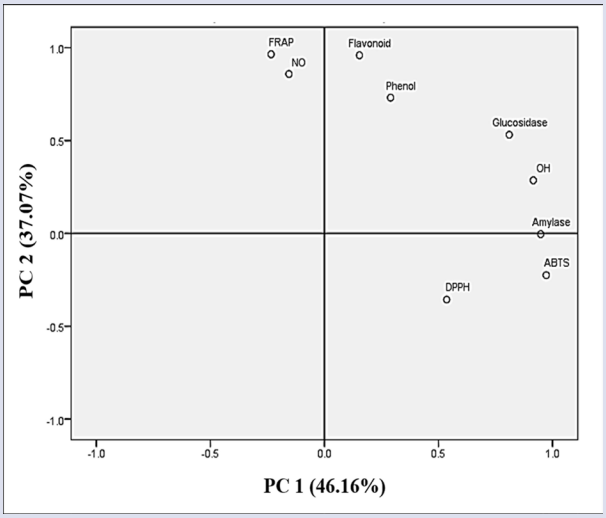

Figure 3: Loading plot of contribution of variables of antidiabetic and antioxidant activities of *L. cubeba* on the bidimensional space formed by rotated principal components 1 and 2.

Table 4: Bioactive components identified from methanol extract of *L. cubeba*.

Sl. no.	Compound names	RT	Peak area (%)	Molecular formula	M_r
1	Butanamide, N,N,3,3-tetramethyl	6.45	0.028	$C_8H_{17}NO$	143.23
2	1,2- Cyclooctanediol	8.68	0.241	$C_8H_{16}O_2$	144.21
3	2-Isopropylidene-5-methylhex-4-enal	10.07	0.369	$C_{10}H_{16}$	152.23
4	7-oxabicyclo(4,1,0)heptan-2-one,3-methyl-6-(1-methylethyl) [carvenone oxide]	11.52	0.710	$C_{10}H_{16}O_2$	168
5	4,7,7-Trimethyl-3,9-dioxatricyclo[6,1,0,0(2,4)]nonan-5-one	12.18	0.497	$C_{10}H_{14}O_3$	182
6	α-methyl- α[4-methyl-3-pentenyl]oxiranemethanol (1,2-oxolinalool)	13	0.653	$C_{10}H_{18}O_2$	170
7	(-)-Spathulenol	13.45	0.81	$C_{15}H_{24}O$	220
8	4H-1-Benzopyran-4-one,7-Hydroxy-2-(4-hydroxyphenyl)	14.62	0.284	$C_{15}H_{10}O_4$	254
9	Isoaromadendrene Epoxide	15.28	0.184	$C_{15}H_{24}O$	220
10	Corymbolone	16.25	0.326	$C_{15}H_{24}O_2$	236
11	1,3,3-Trimethyl-2-oxabicyclo-(2,2,2)octane,6,7-endo, endo-diol	16.97	0.511	$C_{10}H_{18}O_3$	186
12	Estra-1,3,5(10)-trien-17a-ol	17.78	0.781	$C_{18}H_{24}O$	256
13	2-Methoxy-4-[(2-pyridin-4-yl-ethylimino)-methyl]-phenol	18.25	0.255	$C_{15}H_{16}N_2O_2$	256
14	Sandaracopimar-7,15-dien-6-one	18.9	0.326	$C_{20}H_{30}O$	286
15	Oleic acid	19.52	1.506	$C_{18}H_{34}O_2$	282
16	4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl) [Apigenin]	19.83	0.597	$C_{15}H_{10}O_5$	270
17	4H-1- Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy-	21.08	0.213	$C_{17}H_{14}O_6$	314
18	Morin	21.7	0.199	$C_{15}H_{10}O_7$	302
19	4H-1- Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-	24.38	0.056	$C_{17}H_{14}O_7$	330

FRAP assay

FRAP activity of the extracts was expressed in terms of mM of Fe^{+2} equivalent. High reducing activity was observed in case of methanolic extract (0.47 ± 0.023 mM of Fe^{+2} equivalent) followed by ethanol extract (0.44 ± 0.018 mM of Fe^{+2} equivalent). The results were recorded in Table 3.

Nitric oxide scavenging assay

Nitric oxide scavenging activity of the standard and the extracts were shown in Figure 2C. Only methanol and ethanol extracts exhibited the scavenging property at the higher concentrations, as observed by their IC_{50} values. However, these values are comparatively much lower than the standard IC_{50} value of ascorbic acid. No significant scavenging activity was observed in the remaining extracts (Table 2).

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was observed in the following order: Ascorbic acid>ethanol extract>methanol extract> water extract (Figure 2D). The IC_{50} values of the extracts were listed in Table 2. Acetone and hexane extracts did not exhibit satisfactory scavenging activity.

Quantification of antioxidative biochemicals

Total phenol content

Total phenol content of the plant extracts was expressed in terms of mg. of gallic acid equivalent/g. According to the experimental outcome, the total phenol content of the extracts were maximum in methanol extract (182.43 ± 1.23mg. of gallic acid equivalent/g) followed by ethanol extract (120.53 ± 2.79 mg. of gallic acid equivalent/g) and acetone extract (117.41 ± 5.09mg. of gallic acid equivalent/g) as shown in the Table 3. Very less amount of phenolic content was found in hexane extract (1.37 ± 0.4 mg of gallic acid equivalent/g).

Total flavonoid content

Total flavonoid content of the extracts was expressed in terms of mg. of quercetin equivalent/g. The flavonoid content was high in methanol (189.36 ± 5.81mg. of quercetin equivalent/g) and ethanol extract (145.64 ± 2.26mg. of quercetin equivalent/g) compared to the others which were below that values (Table 3).

Principal component analysis

Principal component analysis was applied to evaluate the all the experimental data (Figure 3). PCA results exhibited that the first two principal components (PC1 and PC2) accounted for 83.24 % of the total variability, of which 46.16 % was along the first principal component (PC1), and 37.07 % was along the second principal component (PC2). It was clearly observed that PC1 was positively correlated with α-amylase and α- glucosidase inhibitory activity, hydroxyl and ABTS scavenging activity. FRAP activity and NO scavenging activity was positively correlated with PC2.

GC-MS analysis

The GC-MS analysis of the methanolic extract showed presence of 19 compounds as shown in the Table 4 and the corresponding mass spectrum was shown in Figure 4. The chief compounds in that extract were - Butanamide N,N,3,3-tetramethyl; 1,2- Cyclooctanediol; 2-isopropylidene-5-methylhex-4-enal; 7-oxabicyclo(4,1,0)heptan-2-one,3-methyl-6-(1-methylethyl);4,7,7-Trimethyl-3,9-dioxatricyclo[6,1,0,0(2,4)]nonan-5-one; a´-methyl-a´[4-methyl-3-entenyl]oxirenemethanol; (-)-Spathulenol; 4H-1-Benzopyran-4-one; 7-Hydroxy-2-(4-hydroxyphenyl; Isoaromadendrene Epoxide; Corymbolone; 1,3,3-Trimethyl-2-oxabicyclo-(2,2,2) octane,6,7-endo,endo-diol;Estra-1,3,5(10)trien-17-a´-ol;2-Methoxy-4-[(2-pyridin-4-yl-ethylimino)-methyl]-phenol; Sandaracopimar-7,15-dien-6-one; Oleic acid; 4H-1-Benzopyran-4-one; 5,7-dihydroxy-2-(4-hydroxyphenyl)- (Apigenin); 4H-1- Benzopyran-4-one; 5,7-dihydroxy-2-(4-

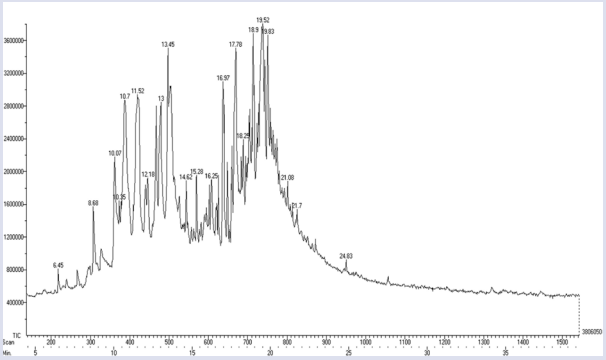

Figure 4: GC-MS profile of methanol extract of *L. cubeba*. Here, the *x*-coordinate, or abscissa represents the scan time (min) and the *y*-coordinate, or ordinate represents the relative abundance in ppm.

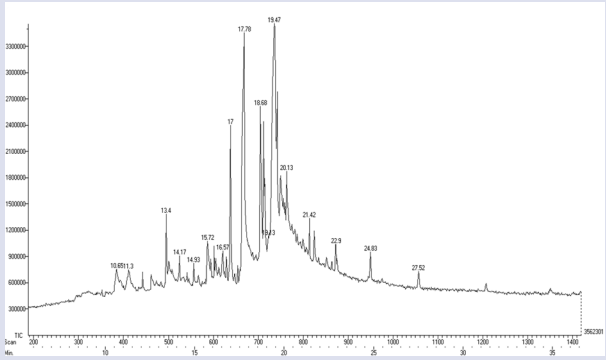

Figure 5: GC-MS profile of ethanol extract of *L. cubeba*. Here, the *x*-coordinate, or abscissa represents the scan time (min) and the *y*-coordinate, or ordinate represents the relative abundance in ppm.

Table 5: Bioactive components identified from ethanol extract of *L. cubeba*.

Sl. no.	Compound names	RT	Peak area (%)	Molecular formula	M_r
1	4-Hepten-3-ol,4-methyl-	10.65	0.929	$C_8H_{16}O$	128
2	2(5H)-Furanone,5-ethyl-3-hydroxy-4-methyl-	11.3	1.014	$C_7H_{10}O_3$	142.75
3	Phenol, 2,4-bis[1,1-dimethylethyl]-	13.4	0.707	$C_{14}H_{22}O$	206
4	Flavone	14.17	0.464	$C_{15}H_{10}O_2$	222
5	Tetradecanoic acid/ Myristic acid	15.72	1.003	$C_{14}H_{28}O_2$	227.67
6	Hexadecanoic acid,Z-11	16.57	0.57	$C_{16}H_{30}O_2$	254
7	Hexadeanoic acid, methyl ester	17	1.119	$C_{17}H_{34}O_2$	270
8	n-Hexadecanoic acid/ palmitic acid	17.78	3.919	$C_{16}H_{32}O_2$	256
9	Phytol	18.68	1.669	$C_{20}H_{40}O$	296
10	Oleic acid	19.47	5.979	$C_{18}H_{34}O_2$	282
11	12-methyl -E,E-2,13-octadecadien-1-ol	20.13	1.912	$C_{19}H_{36}O$	280
12	3,7,3′,4′-tetrahydroxyflavone	21.42	0.76	$C_{15}H_{10}O_6$	286
13	4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-methoxyphenyl)-	22.9	0.729	$C_{16}H_{11}O_5$	312
14	4H-1-Benzopyran-4-one, 5-hydroxy-2(4-hydroxyphenyl)-3,7-dimethoxy-/ Kumatakenin	24.83	0.57	$C_{17}H_{14}O_6$	314
15	Isopropyl stearate	27.52	0.528	$C_{21}H_{42}O_2$	326

hydroxyphenyl)-3,7-dimethoxy-; Morin; 4H-1- Benzopyran-4-one and 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-methoxy-.

Whereas the GC-MS analysis of the ethanolic extract identified 15 compounds (Table 5) which were- 4-Hepten-3-ol,4-methyl-; 2(5H)-Furanone,5-ethyl-3-hydroxy-4-methyl-; Phenol; 2,4-bis[1,1-dimethylethyl]-; Flavone; Tetradecanoic acid; Hexadecanoic acid Z-11, hexadeanoic acid, methyl ester;n-Hexadecanoic acid; Phytol; oleic acid;12-methyl -E,E-2,13-octadecadien-1-ol; 3,7,3′,4′-tetrahydroxyflavone; 4H-1-Benzopyran-4-one; 5,7-dihydroxy-2-(4-methoxyphenyl)-; 4H-1-Benzopyran-4-one; 5-hydroxy-2(4-hydroxyphenyl)-3,7-dimethoxy- and Isopropyl stearate . The mass spectrum of the GC-MS analysis of ethanol extract was shown in the Figure 5.

DISCUSSION

A large number of active secondary metabolites were found to be present in the extracts which might be responsible for its hypoglycemic and antioxidant activities. Phenols and flavonoids were found as the major phytochemicals present in all the extracts which are very remarkable as these compounds are already found to be useful against diabetes-related health issues. Presence of anthocyanins and tannins might also be important contributors of the antioxidative potential of the extracts.

Carbohydrate-degrading enzymes are considered to be one of the major targets in treating hyperglycemia-induced obesity and other metabolic dysfunctions.[6] These digestive enzymes (salivary and pancreatic alpha-amylase, alpha-glucosidase) aid in the breakdown of complex carbohydrates of food and hydrolyze them into smaller units of maltose and glucose, increasing the concentration of simple sugars in the bloodstream.[44] Inhibition of digestive enzymes *viz.* alpha-amylase and alpha-glucosidase can effectively reduce the post-prandial glucose levels in blood in type-2 patients.[45] In our experiments, it was observed that *L. cubeba* (Lour.) Pers. shows promising inhibitory activity upon both the enzymes. Methanol extract exhibited high inhibitory activity against α-amylase as indicated by the IC_{50} value (514.9 µg/mL). The IC_{50} value

of ethanol extract was 1111.1µg/mL, about two-fold of the previous one. However, water extract did not exhibit significant inhibitory activity (IC_{50} = 4235.4 µg/mL). No or very less inhibition was observed in acetone and hexane extracts. It suggests that the possible mechanism of action of this plant lies within its enzyme inhibitory activities, conferring a suitable pharmacological parallelism between the experimental outcome and the documentation of its traditional hypoglycemic virtues.

Diabetes management approaches often focus on the restoration of impaired antioxidative defense. Free radical scavenging activity is considered to be one of the desired criteria for selection of an antidiabetic drug. In this context, several radical scavenging assays were performed to evaluate the antioxidant activities of the plant extracts *in vitro*. DPPH radical is regarded as a model of a stable lipophilic radical and therefore DPPH radical scavenging assay is a widely used evaluative method for antioxidative screening of plant extracts. In this experiment, both ethanol and methanol extracts exhibit high scavenging activity as observed with their IC_{25} and IC_{50} values. Similarly, quenching of $ABTS^{+}$ cation (a proton radical) by the extracts was an important attribute in antioxidative potential. Hydroxyl radical is the highly reactive free radical as it frequently induces DNA damage, cytotoxicity, and carcinogenesis.[46] Moderate scavenging activities of hydroxyl radical by the extracts compared to the standard can also be considered significant in this regard. Excess production of nitric oxide and its reaction with oxygen is responsible for the formation of destructive nitrite and peroxynitrite anions, leading to the oxidative stress development. In our experiment, fruit extracts (ethanol and methanol) showed concentration-dependent scavenging activity on nitric oxide. Moreover, reduction of ferric (Fe^{+3}) ions by the extracts further confirmed its antioxidative power.

Phenols and flavonoids are well known for their immense role as anti-inflammatory and antioxidant compounds. They can donate hydrogen atoms to the reactive free radicals and successfully quench them into less reactive reduced forms.[47] High flavonoid and phenol content were measured in methanol and ethanol extracts, which suggest the possible chemical nature of the antidiabetic active compounds.

From the experimental results, it was observed that methanol and ethanol extracts exhibit significant hypoglycemic and antioxidant activities compared to water and non-polar solvents (acetone and hexane). High phenol and flavonoid content in alcohol extracts also confirmed that the hypoglycemic active compound(s) may be polar or semi-polar in nature. Therefore, partial characterization of the lead molecule(s) by GC-MS analysis was carried out from the alcohol extracts. In this study, Principal component analysis (PCA) was conducted on the correlation matrix for better visualization of the data sets obtained from the determinations of all studied variables. The loading of PC1 and PC2 with the variables explains the correlation coefficients among all the experimental parameters and justifies the antidiabetic screening of the plant extracts along with its antioxidant activities.

GC-MS analysis of both the methanol and ethanol extracts of the fruits share a common metabolite, oleic acid, which is an unsaturated fatty acid present in almost all types of vegetable oils. Oleic acid helps to minimize the inflammatory responses caused by type-2 diabetes. It also regulates insulin secretion and enhances basal utilization of insulin by reversing the inhibitory effect of TNF-α.[48] Immunomodulatory and cardioprotective activities of oleic acid are also reported from various pharmacological studies. Moreover, it possesses an extraordinary attribute in drug absorption.[49] Methanol extract also contains a number of dietary flavones, among them morin exerts insulin-mimetic activities in diabetic model cells.[50] The strong inhibitory activity of morin towards protein-tyrosine phosphatase 1B was also observed which indicates its potent role in sensitizing and activating insulin production as well as anti-obesity attributes, especially in case of insulin resistance.[51] Morin exerts its anti-oxidant activity by chelation of metal ions such as Fe^{+2} ions and also prevent lipid peroxidation in case of myocardial infarction.[52] Scavenging of diabetes-induced reactive oxygen species generation, upregulation of antioxidative genes and prevention of hepatopathy by morin was also reported from several experiments.[53] Other dietary flavones, 4H-1-Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-/ Apigenin and 4H-1- Benzopyran-4-one, 5,7-dihydroxy-2-(4-hydroxyphenyl)-3,7-dimethoxy- also possess significant immunomodulatory, antioxidant and anticancer properties.[54-56] Evidence of insulin-secretagogue activity of apigenin in Diabetic rats was also reported.[57] Phytol and its derivatives have already proved to confer improved glucose tolerance and insulin sensitization.[58] Therefore, a number of bioactive components had been identified from the alcohol extracts that might act together for maintaining the overall glucose homeostasis.

CONCLUSION

The use of synthetic alpha-amylase and glucosidase inhibitors (like acarbose, voglibose, migitol etc.) for the treatment of hyperglycemia imparts some unavoidable impacts on hepatic, gastrointestinal and urinary systems.[59] Therefore, the urge for the adoption of herbal therapy remains the only way-out that could minimize the serious side effects of the synthetic drugs as well as able to satisfy the intervention of glucose homeostasis in diabetic people. The pre-requisites for herbal drug discovery rely mostly up on the primary health care system that is solely dependent up on the traditional knowledge of the ethnic people that is in practice for thousands of years. Taking cue from this, the fruits of *L. cubeba* were selected for assessment of antidiabetic and antioxidant activity, which has been known to prevent and check diabetes among the people of Darjeeling Hills. In our study, the fruit showed potent alpha-amylase and glucosidase activity. Also, the GC-MS analysis revealed the presence of several compounds that may be correlated with the hypoglycemic property of the plant. The remarkable antioxidant property of the fruits may aid in scavenging the deleterious effects of free radicals generated in the body due to high blood sugar level. Therefore, based up on our observations, we could validate the hypoglycemic property of *L. cubeba* fruits that has been in use by the local people of Darjeeling Hills.

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CONFLICT OF INTEREST

None declared by the authors.

ABBREVIATIONS

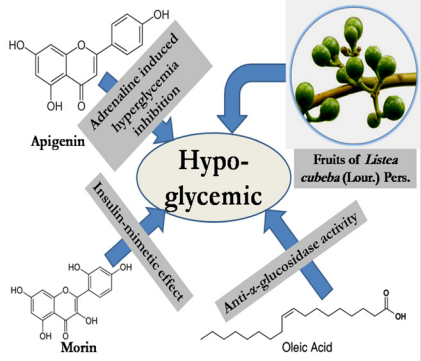
IC_{50}: concentration at which 50% inhibition was observed, **IC25:** concentration at which 25% inhibition was observed, **RT:** retention time, **Mr:** molecular mass, **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl, **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, **TPTZ :** 2,4,6-Tris(2-pyridyl)-s-triazine, **GC-MS:** gas chromatography- mass spectrometry, **NIST:** National Institute of Standards and Technology.

REFERENCES

1. Ezuruike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J Ethnopharmacol. 2014;155(2):857-924.
2. Rask-Madsen C, King GL. Vascular complications of diabetes: mechanisms of injury and protective factors. Cell Metab. 2013;17(1):20-33.

3. Wright E, Scism-Bacon J, Glass L. Oxidative stress in type 2 diabetes: the role of fasting and postprandial glycaemia. Int J Clin Pract. 2006;60(3):308-14.
4. Matheus ASM, Tannus LRM, Cobas RA, Palma CCS, Negrato CA, Gomes MB. Impact of diabetes on cardiovascular disease: An update. Int J Hypertens. 2013;653789.
5. Ullah A, Khan A, Khan I. Diabetes mellitus and oxidative stress - A concise review. Saudi Pharm J. 2016;24(5):547-53.
6. Sales PM, Souza PM, Simeoni LA, Silveira D. α-Amylase inhibitors: a review of raw material and isolated compounds from plant source. J Pharm Pharm Sci. 2012;15(1):141-83.
7. Cefalu WT, Stephens JM, Ribnicky DM. Diabetes and Herbal (Botanical) Medicine. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. 2nd ed. Boca Raton (FL): CRC Press/Taylor and Francis; 2011.
8. Jarald E, Joshi SB, Jain DC. Diabetes and Herbal Medicines. Iran J Pharm Ther. 2008;7(1):97-106.
9. The Plant List (2013). Version 1.1. http://www.theplantlist.org/ (accessed 1st September 2017).
10. Liu TT, Yang TS. Antimicrobial impact of the components of essential oil of Litsea cubeba from Taiwan and antimicrobial activity of the oil in food systems. Int J Food Microbiol. 2012;156(1):68-75.
11. Xie ZW, Yu YQ. The guide of National Chinese Herbal Medicine. Beijing: People's Medical Publishing House. 1996;1.
12. Liao PC, Yang TS, Chou JC, Chen J, Lee SC, Kuo YH, *et al.* Anti-inflammatory activity of neral and geranial isolated from fruits of *Litsea cubeba* Lour. J Funct Foods. 2015;19:248-258.
13. Skaria BP, Joy PP, Mathew S, Mathew G, Joseph A, Joseph R. Aromatic Plants. Vol. 1. Horticulture Science Series. New Delhi, India: New India Publishing Agency. 2007.
14. Yang Y, Jiang J, Qimei L, Yan X, Zhao J, Yuan H, *et al.* The fungicidal terpenoids and essential oil from *Litsea cubeba* in Tibet. Molecules. 2010;15(10):7075-82.
15. Wang H, Liu Y. Chemical composition and antibacterial activity of essential oils from different parts of *Litsea cubeba*. Chem Biodivers. 2010;7(1):229-35.
16. Su YC, Ho CL. Essential oil compositions and antimicrobial activities of various parts of Litsea cubeba from Taiwan. Nat Prod Commun. 2016;11(4):515-8
17. Bayala B, Bassole IHN, Scifo R, Gnoula C, Morel L, Lobaccaro JMA, *et al.* Anti-cancer activity of essential oils and their chemical components - a review. Am J Cancer Res. 2014;4(6):591-607.
18. Feng X, Jiang H, Zhang Y, He W, Zhang L. Insecticidal activities of ethanol extracts from thirty Chinese medicinal plants against Spodoptera exigua (Lepidoptera: Noctuidae). J Med Plants Res. 2012;6(7):1263-7.
19. Gautam N, Mantha AK, Mittal S. Essential oils and their constituents as anticancer agents: A mechanistic view. Bio Med Res Int. 2014;154106.
20. Chen HC, Chang WT, Hseu YC, Chen HY, Chuang CH, Lin CC, *et al.* Immunosuppressive effect of *Litsea cubeba* L. essential oil on dendritic cell and contact hypersensitivity responses. Int J Mol Sci. 2016;17(8):1319.
21. Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA, Anwar F. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed. 2015;5(8):601-11.
22. Wang Y, Jiang ZT, Li R. Antioxidant activity, free radical scavenging potential and chemical composition of *Litsea cubeba* essential oil. J Essent Oil bear Pl. 2012;15(1):134-143.
23. Seal S, Chatterjee P, Bhattacharya S, Pal D, Dasgupta S, Kundu R, *et al.* Vapor of volatile oils from *Litsea cubeba* seed induces apoptosis and causes cell cycle arrest in lung cancer cells. PLoS One. 2012;7(10):e47014.
24. Konsam S, Thongam B, Handique AK. Assessment of wild leafy vegetables traditionally consumed by the ethnic communities of Manipur, northeast India. J Ethnobiol Ethnomed. 2016;12(1):9.
25. Brahma J, Brahma BK. Nutritional and phytochemical evaluation of some wild aromatic plants used as sources of food and medicines by the bodo tribes of Kokrajhar District, Assam, India. Int J Conserv Sci. 2016;7(1):137-46.
26. Namsa ND, Mandal M, Tangjang S, Mandal SC. Ethnobotany of the Monpa ethnic group at Arunachal Pradesh, India. J Ethnobio Ethnomed. 2011;31:1-14.
27. Rajkumari R, Singh PK, Das AK, Dutta BK. Ethnobotanical investigation of wild edible and medicinal plants used by the Chiru Tribe of Manipur, India. Pleione. 2013;7(1):167-74.
28. Chhetri DR, Parajuli P, Subba GC. Antidiabetic plants used by Sikkim and Darjeeling Himalayan tribes India. J Ethnopharmacol. 2005;99(2):199-202.
29. Chakraborty R, Roy S, Mandal V. Assessment of traditional knowledge of the antidiabetic plants of Darjeeling and Sikkim Himalayas in the context of recent phytochemical and pharmacological advances. J Integr Med. 2016;14(5):336-58.
30. Lu YX, Zhang Q, Li J, Sun YX, Wang LY, Cheng WM, *et al.* Antidiabetic effects of total flavonoids from Litsea coreana leaves on fat-fed, streptozotocin-induced type 2 diabetic rats. Am J Chin Med. 2010;38(4):713-25.
31. Sohn E, Kim J, Kim CS, Lee YM, Jo K, Shin SD, *et al.* The extract of Litsea japonica reduced the development of diabetic nephropathy via the inhibition of advanced glycation end products accumulation in db/db mice. Evid Based Comp Alt Med. 2013;769416.
32. Chowdhury A, Rahman MM, Hossain MN, Rahmatullah M. Antihyperglycemic activity of Litsea glutinosa (Lour.) B. Rob. bark methanolic extract. World J Pharm Pharmac Sci. 2017;6(1):209-16.
33. Jeyaseelan EC, Tharmila S, Sathiyaseelan V, Niranjan K. Antibacterial activity of various solvent extracts of some selected medicinal plants present in Jaffna Peninsula. Int J Pharm Biol Arch. 2012;3(4):792-6.
34. Ugochukwu SC, Arukwe UI, Ifeanyi O. Preliminary phytochemical screening of different solvent extracts of stem bark and roots of Dennetia tripetala G. Baker. Asian J Plant Sci Res. 2013;3(3):10-3.
35. Ranilla LG, Kwon YI, Apostolidis E, Shetty K. Phenolic compounds, antioxidant activity and *in vitro* inhibitory potential against key enzymes relevant for hyperglycemia and hypertension of commonly used medicinal plants, herbs and spices in Latin America. Bioresource Technol. 2010;101(12):4676-89.
36. Vinholes J, Grosso C, Andrade PB, Gil-Izquierdo A, Valentão P, Pinho PGD, *et al.* *In vitro* studies to assess the antidiabetic, anti-cholinesterase and antioxidante potential of Spergularia rubra. Food Chem. 2011;129(2):454-62.
37. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181:1199-200.
38. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9-10):1231-7.
39. Benzie F, Strain J. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. Anal Biochem. 1996;239(1):70-6.
40. Garratt D. The quantitative analysis of Drugs. Japan: Chapman and Hall Ltd. 1964;456-8.
41. Yu W, Zhao Y, Shu B. The radical scavenging activities of radix puerariae isoflavonoids: a chemiluminescence study. Food Chem. 2004;86(4):525-9.
42. Singleton V, Rossi J. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic. 1965;16(3):144-58.
43. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999;64(4):555-9.
44. Kazeem MI, Adamson JO, Ogunwande IA. Modes of inhibition of α-amylase and α-glucosidase by aqueous extract of Morinda lucida Benth leaf. BioMed Res Int. 2013;527570.
45. Tundis R, Loizzo MR, Menichini F. Natural products as alpha-amylase and alpha-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem. 2010;10(4):315-31.
46. Safavi M, Foroumadi A, Abdollahi M. The importance of synthetic drugs for type 2 diabetes drug discovery. Expert Opin Drug Discov. 2013;8(11):1339-63.
47. Gutierrez RMP, Baez EG. Evaluation of antidiabetic, antioxidant and antiglycating activities of the Eysenhardtia polystachya. Pharmacogn Mag. 2014;10(2): S404–S418.
48. Vinayagam R, Xu B. Antidiabetic properties of dietary flavonoids: a cellular mechanism review. Nutr Metab. 2015;12(1):60.
49. Vassiliou EK, Gonzalez A, Garcia C, Tadros JH, Chakraborty G, Toney JH. Oleic acid and peanut oil high in oleic acid reverse the inhibitory effect of insulin production of the inflammatory cytokine TNFα both *in vitro* and *in vivo* systems. Lipids Health Dis. 2009;8(1)25.
50. Sales-Campos H, Souza PRD, Peghini BC, Silva JSD, Cardoso CR. An overview of the modulatory effects of oleic acid in health and disease. Mini Rev Med Chem. 2013;13(2):201-10.
51. Gu TT, Song L, Chen TY, Wang X, Zhao XJ, Ding XQ, *et al.* Fructose down regulates miR-330 to induce renal inflammatory response and insulin signaling impairment: Attenuation by morin. Mol Nutr Food Res. 2017;61(8):1600760.
52. Paoli P, Cirri P, Caselli A, Ranaldi F, Bruschi G , Santi A, *et al.* The insulin-mimetic effect of Morin: A promising molecule in diabetes treatment. Biochem Biophys Acta. 2013;1830(4):3102-11.
53. Al-Numair KS, Chandramohan G, Alsaif MA, Veeramani C, El Newehy AS. Morin, A Flavonoid, on Lipid Peroxidation and Antioxidant Status in Experimental Myocardial Ischemic Rats. Afr J Tradit Complement Altern Med. 2014;11(3):14-20.
54. Kapoor R, Kakkar P. Protective Role of Morin, a flavonoid, against high glucose induced oxidative stress mediated apoptosis in primary rat hepatocytes. PLoS One. 2012;7(8):e 41663.
55. Shukla S, Gupta S. Apigenin: A promising molecule for cancer prevention. Pharm Res. 2010;27(6):962-78.
56. Lampropoulos P, Lambropoulou M, Papalois A, Basios N, Manousi M, Simopoulos C, *et al.* The role of apigenin in an experimental model of acute pancreatitis. J Surg Res. 2013;183(1):129-37.
57. Woo JH, Ahn JH, Jang DS, Lee KT, Choi JH. Effect of kumatakenin isolated from cloves on the apoptosis of cancer cells and the alternative activation of tumor-associated macrophages. J Agric Food Chem. 2017;65(36):7893-9.
58. Cazarolli LH, Folador P, Moresco HH, Brighente IM, Pizzolatti MG, Silva FR. Mechanism of action of the stimulatory effect of apigenin-6-C-(2"-O-alpha-l-rhamnopyranosyl)-beta-L-fucopyranoside on 14C-glucose uptake. Chem Biol Interact. 2009;179(2-3):407-12.
59. Elmazar MM, El-Abhar HS, Schaalan MF, Farag NA. Phytol /Phytanic acid and insulin resistance: potential role of phytanic acid proven by docking simulation and modulation of biochemical alterations. PLoS One. 2013;8(1):e45638.

GRAPHICAL ABSTRACT



SUMMARY

- *Litsea cubeba* (Lour.) Pers. has been consumed in raw or pickled form by the local people of Darjeeling Hills as a traditional cure or remedy for hyperglycemia and hyperlipidemia.
- The methanolic and ethanolic extracts of the fruit indicated their hypoglycemic properties in the *in vitro* alpha-amylase and alpha-glucosidase inhibition assays.
- The extracts were also highly potent in scavenging free radicals like DPPH, ABTS etc.
- GC-MS characterization of the extracts indicated the presence of active compounds facilitating the above mentioned properties.
- The present study thus provides an evidence of the hypoglycemic and antioxidant activities of fruits of *Litsea cubeba* (Lour.) Pers.

ABOUT AUTHORS

Rakhi Chakraborty has been working for almost 3 years as a PhD scholar in Plant and Microbial Physiology and Biochemistry Laboratory, University of Gour Banga. Her research interests include the scientific validation of the antidiabetic plants of the Darjeeling Himalaya that has been reported to be effective as traditional medicine.

Vivekananda Mandal is working in the field of Phytochemistry and his main area of focus is to isolate and characterize the lead inhibitory molecules of antimicrobials and antidiabetics.

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