Evaluation of *in vitro* antidiabetic and antioxidant characterizations of *Elettaria cardamomum* (L.) Maton (Zingiberaceae), *Piper cubeba* L. f. (Piperaceae), and *Plumeria rubra* L. (Apocynaceae)

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Abstract: Inhibition of intestinal α-amylase and α-glucosidase is an important strategy to regulate diabetes mellitus (DM). Antioxidants from plants are widely regarded in the prevention of diabetes. Fruits of *Elettaria cardamomum* (L.) Maton (Zingiberaceae) and *Piper cubeba* L. f. (Piperaceae) and flowers of *Plumeria rubra* L. (Apocynaceae) are traditionally used to cure DM in different countries. However, the role of these plants has been grossly under reported and is yet to receive proper scientific evaluation with respect to understand their traditional role in the management of diabetes especially as digestive enzymes inhibitors. Hence, methanol and aqueous extracts of the aforementioned plants were evaluated for their *in vitro* α-glucosidase and α-amylase inhibition at 1 mg/mL and quantification of their antioxidant properties (DPPH, FRAP tests, total phenolic and total flavonoids contents). *In vitro* optimization studies for the extracts were also performed to enhance *in vitro* biological activities. The % inhibition of α-glucosidase by the aqueous extracts of the fruits of *E. cardamomum*, *P. cubeba* and flowers of *P. rubra* were 10.41 (0.03), 95.19 (0.01), and -2.92 (0.03), while the methanol extracts exhibited % inhibition 13.73 (0.02), 92.77 (0.01), and -0.98 (0.01), respectively. The % inhibition of α-amylase by the aqueous extracts were 82.99 (0.01), 64.35 (0.01), and 20.28 (0.02), while the methanol extracts displayed % inhibition 39.93 (0.01), 31.06 (0.02), and 39.40 (0.01), respectively. Aqueous extracts displayed good *in vitro* antidiabetic and antioxidant activities. Moreover, *in vitro* optimization experiments helped to increase the α-glucosidase inhibitory activity of *E. cardamomum*. Our findings further justify the traditional claims of these plants as folk medicines to manage diabetes, however, through digestive enzymes inhibition effect.

Keywords: *Elettaria cardamomum*, *Piper cubeba, Plumeria rubra,* antidiabetic activity, α-glucosidase, α-amylase, antioxidant activity, optimization study.

INTRODUCTION

Diabetes mellitus (DM) is a typical common syndrome, manifested by hyperglycaemia occurring due to failing in insulin secretion and its action in human body (World Health Organization (WHO), 2012). It is a metabolic disease categorized by prolonged hyperglycaemia (Nickavar & Yousefian, 2011). Chief cause of morbidity and mortality in DM is due to various biochemical deficiencies associated with micro- and macro vascular impairments (Xie *et al.,* 2011; Berger *et al*., 1999). With the continuous increase of diabetic patients across world, the international and national health care agencies have been orchestrating different strategies to combat and manage this disorder efficiently (WHO, 2008). DM is widely considered to be one of the world's main mortalities in next 25 years (International Diabetes Federation (IDF), 2006; King *et al.,* 1998; Zhang *et al.,* 2010), generally upsetting the people in Africa and Asia, where DM proportions are expected to upsurge by two to three times by the end of 2030 (Shaw *et al.,* 2010). Henceforth, the occurrence of DM is increasing worldwide; this urges the need for more new treatment, even though insulin usage and other contemporary remedies can rectify several chronic conditions of diabetes effectively, various impediments or side effects such as hypoglycaemia, flatulence, diarrhoea, abdominal pain and hepatotoxicity are conjoint episodes of the DM. Drug resistance to diabetes medications after prolonged treatment is another unfortunate scenario and chronic problem to unravel. For that reason, however, in spite of the presently existing modern medications, several folkloric and plant based remedies have been endorsed for the successful management of diabetes (WHO, 2012).

Herbs have always been the base for the efficacious cure of several diseases in traditional medicine systems; they have been used throughout the world for the management of diabetes as well (Umar *et al.,* 2010; Yankuzo *et al.,* 2011). Plants based natural medicines have been reported to reveal anti-diabetic activity through different mechanisms of actions. Currently used anti-diabetic drugs

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manifest their anti-diabetic effect through several different mechanisms. One of the mechanisms is by controlling post-prandial glucose in blood by retarding the action of digestive enzymes. Several traditional medicinal plants have been scientifically reported to exhibit α-glucosidase and α-amylase inhibition activity by impeding glucose absorption through inhibiting the action of digestive enzymes on the hydrolysis of complex carbohydrates, thereby, facilitate preventing the high postprandial glucose in the blood. Hence, inhibition of digestive enzymes helps considerably to regulate high blood glucose levels in type II diabetic (T2DM) patients particularly through reducing post-prandial hyperglycemia (PPHG) (Cheplick *et al*., 2010; Tunna *et al*., 2015).

Inhibition of the digestive enzymes has been clearly linked with antioxidants specially flavonoids and phenolic compounds (Wang *et al*., 2012). Natural antioxidants (i.e. phenolic compounds, flavonoids etc.) have been shown to avert *in vivo* oxidative strain, thereby, reducing the onset and prognosis of diseases like diabetes mellitus (Djeridane *et al*., 2015; Cioanca *et al*., 2015). Oxidative stress is the key incidence during diabetes and has been suggested to be responsible for various kinds of tissue damages in diabetics. Extremely reactive species like free radicals have been often associated with the oxidative stress and pathophysiology of diabetes which may be linked together through a common pathway and different sorts of mechanisms for the diabetes impediments i.e. retinopathy, neuropathy, nephropathy and vascular dysfunctions (Al-Qirim *et al*., 2002; Kaleem *et al*., 2006; Ahmed *et al*., 2012; Taher *et al*., 2015). Plants antioxidants have been reported to prevent the development of diabetes mellitus by decreasing the antioxidant level (Kaleem *et al*., 2006; Umar *et al*., 2010), therefore, measurement of antioxidant activity of the plants could be carried out using DPPH test, FRAP test and screening the total phenolic content (TPC) and total flavonoid content (TFC) with respect to know the association of oxidative stress in the manifestation of pancreatic beta-cell impairment in T2DM and to assess the prospective effectiveness of antioxidants in the prevention of T2DM. Several research studies have vividly postulated that antioxidant treatment has been found to display favorable effects in diabetes through the protection of *in vivo* pancreatic beta-cell function (Kaleem *et al*., 2008; Yankuzo *et al*., 2011). The natural antioxidants, apart from preventing oxidative stress have also been reported to reveal *in vivo* digestive enzymes inhibitory activities (Wang *et al*., 2012; Djeridane *et al*., 2015; Tunna *et al*., 2015).

Finding a suitable inhibitor of α-glucosidase and α-amylase with minimal side effects is a current demand and challenge with respect to find out efficacious antidiabetic drug with minimum or no deleterious effects. In regard to tackle this issue effectively, WHO has recommended for the explorations and development of safer and better antidiabetic agents from the natural sources particularly from plants (WHO, 2012). In consideration of this background, selected traditional antidiabetic plants were taken into consideration to accomplish this study. Fruits of *Elettaria cardamomum* (L.) Maton (Zingiberaceae) (Cardamom) and *Piper cubeba* L .f. (Piperaceae) (Cubab) are traditionally used to treat diabetes in Pakistan (Ahmad *et al*., 2009), Bangladesh and Taiwan (HMRC, 2002), respectively. However, the flowers of *Plumeria rubra* L. (Apocynaceae) (Frangipani) are traditionally used in the management of diabetes in Mexico (Adolfo & Heinrich, 2005; Jarald *et al*., 2008). Phytochemical investigations for cardamom have been reported to reveal the presence of various secondary metabolites viz. subinene, terpineol, heptane, phellandrene, limonene, myrcene, cineol, menthone, pinene, pinene (Shaban *et al*., 1987), phytol, sitostenone, eugenyl acetate, sitosterol (Gopalakrishnan *et al*., 1990), nerolidol, linalol (Okugawa *et al*., 1988), stigma sterol, citronellol, p-cymene, bisabolene, borneol, geraniol, geranyl acetate and terpinene (Duke, 1992). However, phytochemical investigations for frangipani have been reported to reveal the presence of tannins, flavonoids, terpenoids, reducing sugar, alkaloids (Egwaikhide *et al*., 2009), 2-methylbutan-1-ol, β-phenylethyl alcohol, nanodecane, heneicosane and benzyl salicylate (Sulaiman *et al*., 2008). Phytochemical screening for cubab has been reported to contain alkaloids, glycosides, steroids, flavonoids, tannins and anthraquinones (Nahak & Sahu, 2011).

These findings prompted us to carry out current study to investigate the antioxidant and antidiabetic properties through detecting the total flavonoid content (TFC), phenolic content (TPC), radical scavenging activity (DPPH), ferric-reducing antioxidant power (FRAP) method, α-amylase and α-glucosidase inhibitory activities of aforementioned three traditional medicinal plants widely used to manage diabetes across the world. Since in scientific literature, no report exists for *in vitro* α-amylase and α-glucosidase inhibitory activities of these plants. Hence, this research was aimed to evaluate the inhibitory effects of the aforementioned plants against α-glucosidase and α-amylase enzymes as well as to determine their antioxidant characteristics by using *in vitro* methods.

MATERIAL AND METHODS

Chemical and enzymes

Porcine pancreatic α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) enzymes from Baker's yeast, *p*-nitrophenyl-α-D-glucopyranoside, 3,5-dinitrosalicylic acid, starch potato soluble, DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin & Ciocalteu's phenol reagent, gallic acid and 2,4,6-tri (2-pyridyl)-*s*-triazine (TPTZ) were

bought from Sigma Aldrich. Aluminum chloride was purchased from Fisher, Malaysia.

Plant materials

Fruits of *Elettaria cardamomum* L. (Cardamom) and *Piper cubeba* L. (Cubab) were purchased from OXL resources Sdn. Bhd, Malaysia. Fresh flowers of *Plumeria rubra* L.(Frangipani) were collected from IIUM Kuantan campus, Malaysia in April 2012 and the specimens were authenticated by the taxonomist (Kulliyyah of Pharmacy, IIUM). The voucher specimens of all the three plants have been deposited in the Herbarium, Kulliyyah of Pharmacy, IIUM, Kuantan, Pahang DM, Malaysia for future references.

Extraction: Preparation of aqueous (AQ) and methanol (MeOH) extracts

Fresh flowers of frangipani and fruits of cardamom and cubab were dried in a laboratory dryer (30-40 °C) and then pulverized to a coarse powdered using universal cutting mill (Schemersal, Garmany). Powdered plant materials were soaked at room temperature in two different solvents (i.e. distilled water (H_2O) and analytical grade (AR) methanol) in a tightly closed round bottom flask for 72 hours and carefully filtered using Whatman filter paper. The entire extraction method was repeated thrice to ensure maximum yield and the extracts were freeze dried until further analysis.

Anti-diabetic activity in vitro

All measurements were conducted in triplicates. All six extracts from three plants (*E. cardamomum* L., *P. cubeba* L. and *P. rubra* L.) were evaluated for their inhibitory effect towards α-amylase and α-glucosidase at 1 mg/mL concentration (solvent 10% ethanol).

In vitro α-glucosidase inhibition study

In vitro α-glucosidase inhibition study was done by adopting the method described by Marcia Da *et al*. (2008) with slight modification. Briefly, 100 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase enzyme solution (1.0 U/mL) and 50 µL of sample solution were incubated at 25 °C for 10 min in a 96 wells micro plate. After pre-incubation, 50 µL of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH 6.9) was added to every well at 5 seconds intermissions. The solutions were again put for incubation for 5 min at 25 °C in a 96 wells micro plate. After incubation, micro plate reader instrument (Tecan, Switzerland) was used to measure absorbance at 405 nm and the resultant readings were equated to control, which was containing 50 µL of buffer solution instead of plants extracts. The inhibitory action (expressed as inhibition %) for α-glucosidase enzyme was measured as follows:

Inhibition (%) = [(Control 405 - Extract 405) /Control 405] × 100

In vitro α-amylase inhibition study

The experiment to investigate α-amylase inhibitory effect was accomplished by adopting the assay previously carried out successfully by Apostolidis *et al*. (2007) with a little modification to improve analysis further. Briefly, 25 µL of 20 mM phosphate buffer (pH 6.9) containing porcine α-amylase at a concentration of 0.5mg/mL and 25 µL of plants extracts, were incubated at 25 °C for 10 min in a 96 wells micro plate. After pre-incubation, 25µL of 0.5% starch solution in 20 mM phosphate buffer (pH 6.9) was added in a 96 wells micro plate. The solutions (reaction mixtures) were again incubated for 10 min at 25 °C in a 96 wells micro plate. The reaction was forced to stop through the addition of color reagent i.e. 50 µL of 96 mM 3,5-dinitrosalicylic acid (DNS). Afterward, the micro plate was placed on the hot water bath for 5 min and then carefully allowed to cool at room temperature. 100% enzyme's activity was represented by the incubation of control and was done similarly by replacing extracts with vehicle (solvent). For blank incubation (to allow for absorbance to be produced by the plant extract), enzyme solution was substituted by buffer solution and then at 540 nm, absorbance (A) was measured. The inhibitory action (expressed as inhibition %) for α-amylase enzyme was measured as follows:

Inhibition (%) = [(A540Control - A540 Extract) /A540 Control] × 100

In vitro antioxidant activity

All measurements were conducted in triplicates. All six extracts from three plants were evaluated for their antioxidant activity at 1 mg/mL (solvent 10% ethanol).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) method

Free radical scavenging activity of all the extracts was assessed through the assay previously mentioned and done by Kuda and Ikemori (2009) to determine DPPH radical-scavenging effect with slight modification. Briefly, sample solution (0.025mL), distilled water (0.075 mL) and methanol (0.1mL) were put into a 96-wellsmicroplate and 1 mM DPPH in ethanol solution (0.025mL) was added. Mixture of sample was put for incubation for 30min at 37°C in the dark and its absorbance was recorded at 517 nm. Standard represented the solution containing DPPH only without any plant extracts. The free radical scavenging property was stated as percentage inhibition for DPPH scavenging and calculated as follows:

$$\text{DPPH scavenging (\%)} = \frac{OD_{blank} - OD_{sample}}{OD_{blank}} \times 100\%$$

Ferric reducing antioxidant power (FRAP) method

The FRAP experiment was done by following the assay mentioned by Benzie and Strain (1996). Briefly, the ferric-reducing antioxidant power reagent was initially made by mixing 20mM $FeCl_3.6H_2O$, 10mM TPTZ and

300 mM acetate buffer (pH 3.6) in a ratio of 1:1:10. FRAP reagent (180µL) was transferred into test tubes through micropipette and 20µL test solution (1 mg/mL) was added. The absorbance was observed for 4 min at 593 nm. Absorbance is directly proportional to the combined ferric reducing/antioxidant power (FRAP value) for antioxidants presence in the plant sample. Its results were mentioned as mM of Fe (II)/l and were assessed by taking aqueous $FeSO_4.7H_2O$ (0.1-1.0 mM) as standard for calibration (Patthamakanokporn *et al*., 2008; Perez-Jimenez *et al*., 2008).

Test for phenols

Ferric chloride test

Ferric chloride ($FeCl_3$) was used for determining the presence (or absence) of phenols. Little amount of extract was dissolved in 5% ferric chloride solution; Formation of intense color was indicative of the presence of phenols in plant samples (Khan *et al*., 2010).

Folin-Ciocalteau's Method (TPC)

Total phenolic content (TPC) of all the three plant extracts (three aqueous and three methanol extracts) was estimated through Folin-Ciocalteau method (Umar *et al*., 2010). Briefly, in 15 mL falcon tube, 2370 µL of double distilled water, 30 µL of plant's sample and 150 µL Folin-Ciocalteu reagent were taken together and eventually vortexed. Following 1 min, 450 µL of aqueous sodium carbonate (20%) was immixed and later the reaction mixture was again vortexed and put to leave undisturbed for 30 min at 40 °C before taking its absorbance (λ_{max}) at 750 nm. All measurements were done thrice. The TPC concentration was estimated from the calibration curve by taking gallic acid (standard) and the final results were evaluated as mg L^{-1} of gallic acid equivalents (GAE mg L^{-1}).

Test for Flavonoids

Ferric chloride test

The plants samples were mixed with few drops of 5% ferric chloride solution with respect to confirm about the presence of flavonoids. The formation of blackish green color is an indicative of the presence of flavonoids (Khan *et al*., 2010).

Total flavonoid content (TFC)

The TFCs of plants samples were estimated through aluminum chloride assay (Umar *et al.,* 2010). Briefly, 200 µL of water and 100 µL of plant extract (1 mg/mL) were taken together in a 10 mL volumetric flask. Later, after 5 min, 30 µL of 10% aluminum chloride and 300 µL of 5% sodium nitrite were added. Subsequently after 6 min, 200 µL of 1 M sodium hydroxide was immixed and the total volume was made up to 1mL with distilled H_2O. Absorbance of all the samples was estimated and recorded at 510 nm. The total flavonoid content's percentage was estimated through the calibration curve of quercetin plotted by using the similar method and total flavonoid content was mentioned as quercetin equivalents in milligrams/gram plant sample (Ordonez *et al*., 2006).

Optimization of extraction process conditions

A statistical optimization study was done for extraction process condition to obtain maximum value of α-glucosidase inhibition as compared to α-amylase inhibition. From the preliminary stage of the study, plant extracts were selected for optimization study. The process variables used were reaction of incubation time (days), temperature (°C), and volume of solvent (mL)(table 1). In the study, the variables were inspected at three different levels; low (-1), basal (0) and high (+1). The design led to 20 sets of experiment (runs), 7 runs in the focal point. The runs were done in a random order to minimize the effects of the uncontrolled factors. The results from the experimental runs were fitted to a quadratic model shown by equation below:

$$Y= \beta 0 + \beta 1A+ \beta 2B+ \beta 3C+ \beta 11A2+ \beta 22B2+ \beta 33C2+ \beta 12AB + \beta 13AC+ \beta 23BC$$

Where, *Y* is the enzyme inhibition response, while A, B, C are the coded variables for time, temperature and solvent volume, respectively. The constant *β0* refers to the intercept coefficient (regression coefficient at centre point); *β1, β2, β3* are the terms for the single variable effects (linear coefficients); *β11, β22, β33* coefficients predict the double actions of the each factor (quadratic coefficients); and *β12 β13 β23* coefficients indicate the extent of interactions between the variables studied (second order interaction coefficients). A central composite design (CCD) under response surface methodology (RSM) using Design Expert v.6.0.8 (Stat-Ease Inc. Minneapolis) was followed with respect to determine the maximum productivity of α-glucosidase and α-amylase inhibition by illustrating the nature of the response surface in the investigational region and explicate the optimal situations (conditions) of the most significant independent variable (Table 2).

Combination of the extracts

Extracts were mixed to estimate the effect of combined extracts on the antidiabetic and antioxidant activities *in vitro*, the table 3 shows the mixing percentages of the aqueous extracts of the three plants, *E. cardamomum* (cardamom), *P. cubeba* (cubab) and *P. rubra* (frangipani).

STATISTICAL ANALYSIS

Three replicates of every sample were used for statistical analysis of the data generated throughout all experiments. Original data was analyzed through one-way analysis of variance (ANOVA) by using SPSS software 20. P values (<0.05) were taken as significant, results were stated as mean (SD). Also optimization by central composite design (CCD) was carried out with three-process

condition such as temperature, time, and solvent and analyzed by design expert software.

RESULTS

The six crude extracts of three different traditional medicinal plants were evaluated for their inhibitory properties on α-glucosidase and α-amylase at 1 mg/mL concentration, the percentage inhibition was presented as mean (SD). The *in vitro* α-amylase and α-glucosidase inhibitory studies revealed that *E. cardamomum* (cardamom) *and P. cubeba (cubab)* had α-glucosidase inhibitory activity for both of their extracts, while *P. rubra (*frangipani) had negative effect for both of its extracts. The three plants had α-amylase inhibitory activity for both of their extracts (table 4). The highest α-glucosidase inhibitory activities (%) were shown in both of the *cubab extracts.* While the highest α-amylase inhibitory activities (%) were shown in both of the cardamom extracts (fig. 1).Antioxidant activity of aqueous and methanol extracts of all three plants were investigated by their ability to scavenge free radicals by using DPPH assay. Aqueous extract of *cubab* showed significant antioxidant activity *in vitro* in scavenging DPPH radical by 93.53%, followed by cardamom (82.6%) and frangipani (58.05%). For the methanol extracts*,* cubab scavenged DPPH radical by93.13% followed by cardamom by 72.47% and frangipani by 50.85%. Moreover, the reducing potential of all the extracts were also estimated through ferric reducing antioxidant power (FRAP) assay. The results were stated as mM of Fe(II)/l and were assessed using aqueous $FeSO_4$.$7H_2O$ (0.1-1.0 mM). Among the plants*, cubab's* aqueousand methanol extracts gave the highest resultswith 35.46 mM and 33.98 mM FRAP equivalent, respectively. The preliminary screening of the extracts by ferric chloride test revealed about the occurrence of phenolic and flavonoid constituents in appreciable quantity. Therefore, the total phenolic and total flavonoid contents were examined. Result vividly demonstrated that cubab had the highest total phenolic content (TPC) followed by frangipani and cardamom for both methanol and aqueous extracts in term of gallic acid equivalent. Moreover, flavonoid content was found to be the highest in *cubab*, followed by cardamom and frangipani aqueous and methanol extracts.

All the *in vitro* optimization experiments were found to be affected by incubation time. *In vitro* optimization experiments helped to increase the α-glucosidase inhibitory activity of *E. cardamomum*. Its α-glucosidase and α-amylase inhibitions were considerably affected by varying the incubation time and temperatures. Moreover, the combination of the three aqueous extracts of all the tree plants was performed and cubab was found to display the best and most consistent results. The extracts were mixed with respect to find their effects whether they can improve the activity as compared to if they were given alone. Cubab, and cubab mixed extracts showed the best design to increase the activity.

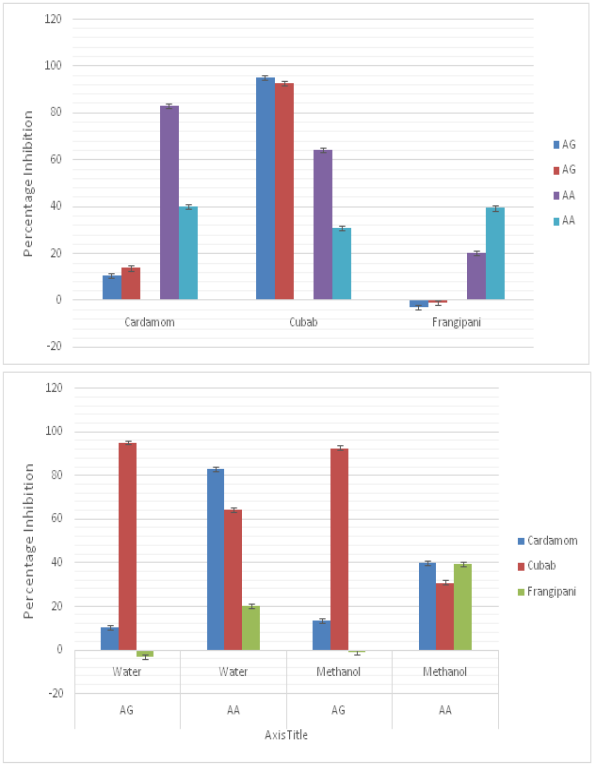

Fig. 1: Inhibitory activity of extracts against α-glucosidase (AG) (1 U/mL) and porcine α-amylase (AA) (1 mg/mL).

DISUSSION

Choice of right solvents is a key orchestrated strategy for extracting bioactive agents (i.e. antioxidants or digestive enzymes inhibitors etc.) from natural sources. Extraction is generally carried out using at least one very polar organic solvent (MeOH or EtOH) followed by distilled water to ensure maximum yield of bioactive substances from plant material (Alam *et al*., 2013). These solvents are considered best for the extraction of all kinds of

Table 1: Experimental range and level of independent process variables

	Name	UNIT	Level of Independent Variables		
			-1	0	1
A	Time [A]	Days	1	3	5
B	Temperature [B]	°C	20	30	40
C	Volume of solvent extraction [C]	mL	10	15	20

bioactive compounds from the plant material simultaneously. In our study, methanol and distilled water were found to be the best solvents to extract plant materials. Several research studies have already proved the efficacy of MeOH/EtOH to ensure maximum yield of biologically active compounds (i.e. low molecular weight molecules, aglycones, etc.) from plant material (Umar *et al.,* 2010; Yankuzo *et al.,* 2011). Other remaining highly polar compounds (i.e. carbohydrates, proteins, tannins, glycones etc.) are easily extracted with distilled water upon slight heating on the water bath, thereby, confirming that all kinds of biologically active compounds are successfully extracted out from the plant material. MeOH has the tendency to extract non-polar, semi polar as well as polar compounds altogether from the plant material upon reflux on the hot water bath and remaining very polar compounds (MeOH/EtOH insoluble compounds) of the plant material are eventually extracted out with distilled water. Hence, aqueous and methanol extracts of all the three plants were selected in this study to ensure that the right extracts containing all the bioactive compounds of plants are isolated properly then investigated correctly for their true pharmacological potential.

Our investigation provides the first *in vitro* evidence of antidiabetic property of extracts of cubab, cardamom and frangipani in the form of digestive enzymes inhibitors. Cubab showed to be a potent inhibitor of α-glucosidase by exhibiting 95.19% inhibition of the enzyme ($p<0.05$) in aqueous extract. Cardamom showed inhibitory activity on α-glucosidase as it demonstrated 10.41%, and frangipani showed a negative inhibition -2.92 ($p<0.05$), this might mean that frangipani may activate or stimulate the digestive enzymes. Cardamom showed the best inhibitory activity on α-amylase enzyme, followed by cubab and frangipani in aqueous extracts ($p<0.05$). Digestive enzymes inhibition experiments for plants extracts outline the inhibitory capability of the plant samples against the enzymes and it is one of the mechanisms through which a plant might reveal its antidiabetic characteristic (Ahmad *et al*., 2009; Tunna *et al*., 2015). Stronger inhibition of α-glucosidase as compared to α-amylase for *P. cubeba* (cubab) is of pronounced pharmacological significance in addressing some of the untoward toxic effects related to the excessive pancreatic α-amylase inhibition (eg. abdominal distention) as earlier studies mentioned that excessive inhibition of α-amylase might be responsible for the unusual bacterial fermentation of undigested carbohydrates in the intestine, hence, slight α-amylase inhibitory property is beneficial (Horii *et al*., 1986). The plants based α-amylase and α-glucosidase inhibitors

Table 2: Experimental design for optimization

Std.	Run	Variables		
		[A] (Days)	[B] (°C)	[C] (mL)
16	1	3	30	15
15	2	3	30	15
19	3	3	30	15
9	4	1	30	15
17	5	3	30	15
14	6	3	30	20
7	7	1	40	20
18	8	3	30	15
8	9	5	40	20
3	10	1	40	10
1	11	1	20	10
5	12	1	20	20
13	13	3	30	10
6	14	5	20	20
2	15	5	20	10
11	16	3	20	15
20	17	3	30	15
4	18	5	40	10
12	19	3	40	15
10	20	5	30	15

suggest a prospective healing and therapeutic approach for the management of post-prandial hyperglycemia (McCue *et al*., 2005). Hence, this might be one of the possible mechanisms for the antidiabetic property of this plant and could be helpful in the management of diabetes mellitus.

Digestive enzymes inhibitors (i.e. acarbose and acarbose like drugs) that retard the effect of α-glucosidase present in the small intestine's epithelium have already been verified to reduce post-prandial hyperglycaemia (Sima & Chakrabarti, 2004) and enhance weakened metabolism of glucose without rectifying secretion of insulin in patients suffering from T2DM (Carrascosa *et al.*, 2001). Such remedies have been acknowledged beneficial for those who have initial stage T2DM as well as for those whose blood glucose levels are slightly higher than the level which is stated perilous for diabetes mellitus. These medications are also regarded advantageous for diabetics consuming metformin or sulfonylurea based antidiabetic drugs as well as for those who are dependent on extra prescriptions in order to control their blood glucose levels within a non-toxic range. Hence, α-glucosidase inhibitors from the natural sources particularly plants propose a potential beneficial strategy for the efficacious prevention of T2DM and borderline patients through decreasing or delaying carbohydrate absorption (McCue *et al.*, 2005).

In-vitro antioxidant activity

Oxidative injury or cellular damage occurs when there is a formation of free radicals appears to be the chief mechanism responsible for human neurodegenerative syndromes such as inflammation, cancers and diabetes (Perez-Jimenez *et al*., 2008). The body produces antioxidants that scavenge these extremely reactive free radical species; however, in the body, the antioxidant guard systems only work efficiently when physiological level of the free radicals is normal or in the safe range (Masoko *et al*., 2005). It has been comprehensively reported through several research studies that oxidation is very much related to the manifestation of DM (Al-Qirim *et al*., 2002; Kaleem *et al*., 2006; Ahmed *et al*., 2012; Taher *et al*., 2015). Moreover, plants possess several free radical scavenging substances i.e. flavonoids (polyphenolic compounds), anthocyanins, saponins, tannins, phenolic acids (phenolic compounds), alkaloids (nitrogen bearing compounds), quinones and carotenoids (terpenes) (Zheng & Wang, 2001), hence, based on the occurrence of such antioxidants in plants abundantly, the antioxidant activity of all three plants extracts were measured through *in vitro* assays.

As shown in table 5*,* six tested plants extracts showed good activity. *P. cubeba* exhibited the highest activity. These plants extracts demonstrated significant results ($p<0.05$). However, some disparities and differences in antioxidant activity and total phenolic and flavonoid contents were also found in examined crude extracts of all the three plants. It can be detected that TPC and TFC in the extracts were found to be greatly associated with their antioxidant activity, confirming that phenolic and flavonoid constituents contribute significantly to the antioxidant property of these plants extracts. As revealed through most of our results, the higher the DPPH, FRAP, phenolic and flavonoid contents, the higher the enzyme inhibitory activity unravelled. The results of this study are in parallel and in good agreement with other similar research studies, which also reported high antioxidant property for the plant extracts showing *in vitro* α-amylase and α-glucosidase inhibitory properties (Esra *et al*., 2004; McCue *et al*., 2005).

Table 3: Mixing of extracts

Extracts mixing percentage	Elaboration
1+2 (0%)	0% cardamom (100% cubab)
1+2 (25%)	25% cardamom (75% cubab)
1+2 (50%)	50% cardamom (50% cubab)
1+2 (75%)	75% cardamom (25% cubab)
1+2 (100%)	100% cardamom (0% cubab)
2+3 (0%)	0% cubab (100% farngipani)
2+3 (25%)	25% cubab (75% farngipani)
2+3 (50%)	50% cubab (50% farngipani)
2+3 (75%)	75% cubab (25% farngipani)
2+3 (100%)	100% cubab (0% farngipani)
1+3 (0%)	0% cardamom (100% farngipani)
1+3 (25%)	25% cardamom (75% farngipani)
1+3 (25%)	50% cardamom (50% farngipani)
1+3 (75%)	75% cardamom (25% farngipani)
1+3 (100%)	100% cardamom (0% farngipani)
1+2+3	Cardamom +cubab +frangipani in equal concentrations

Table 5: DPPH, FRAP, TPC and TFC activates of plants extracts

	DPPH (% Scavenging activity)		FRAP (mM of Fe(II)/l)		TPC (mg gallic acid equivalent)		TFC (quercetin equivalents in mg/g)	
Plants	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Elettaria cardamomum L. (cardamom)	82.60 ±0.05	72.47 ±0.02	11.04 ±0.06	10.59 ±0.01	11.99 ±0.04	6.46 ±0.07	21.02 ±0.06	20.55 ±0.02
*Piper cubeba L. (*cubab)	93.53 ±0.01	93.13 ±0.01	35.46 ±0.04	33.98 ±0.01	91.26 ±0.03	22.14 ±0.01	59.65 ±0.04	49.78 ±0.02
*Plumeria rubra L. (*frangipani)	58.05 ±0.04	50.85 ±0.07	5.84 ±0.01	4.84 ±0.01	5.89 ±0.03	5.39 ±0.01	6.46 ±0.04	7.67 ±0.01

* Mean ±SD

Table 6: Summary of the four optimization experiments

Source	Mean Square	F Value	p-value (Prob > F	R-Squared
Model Y1	319.284	10.3635	0.0005 **	0.903168
Model Y2	590.276	14.9498	0.0001 **	0.93082
Model Y3	231.756	11.4709	0.0004 **	0.9117
Model Y4	85.63	9.92	0.0007 **	0.8993

* p<0.05 specify that the model terms are significant and **p<0.01 specify that the model terms are highly significant.

In vitro optimization study

Stronger inhibition of α-glucosidase as compared to α-amylase is of pronounced pharmacological importance in addressing some of the untoward effects associated with excessive pancreatic α-amylase inhibition (e.g. abdominal distention). This could be the probable mechanism of the antidiabetic activity of these plants and might help to be considered in the management of diabetes mellitus. Hence, *E. cardamomum* was further optimized due to its appreciable α-glucosidase activity in hope to get a better inhibition activity, *P. cubeba* was also further optimized along with cardamom to see whether the optimization could be responsible to increase the inhibition activity. Optimization for the α-amylase inhibition activity was also carried for the two plants in order to relate and compare the effect to the α-glucosidase inhibitory effect. Both plants were also selected due to their good antioxidant activities; as extracts of these two plants showed good DPPH and FRAP activities especially the aqueous extracts. Also the TPC and TFC were higher in these two plants especially in aqueous extracts as compared to methanol extracts. Hence, aqueous extracts were used for further optimization due to above reasons and due to many other reasons including the non-toxic nature of the water and its high polarity nature as the high polar solvent helps to extract bioactive agents easily that are needed for the manifestation of anti-diabetic and antioxidant effects. Moreover, in Ayurvedic system of prescription, a water decoction of medicinal plants is one of the most efficacious ways to cure different ailments (Parasuraman*et al*., 2014).

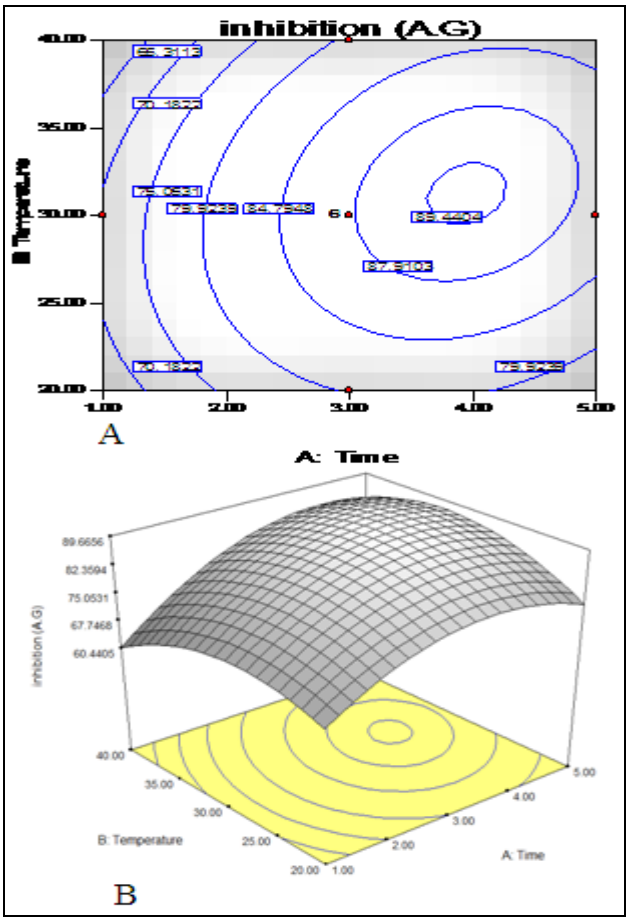

Fig. 2: (a) 2D contour plot and (b) 3D response surface show the effect of incubation time and temperature, and their mutual interaction on percentage of α-glucosidase inhibition by *Piper cubeba.*

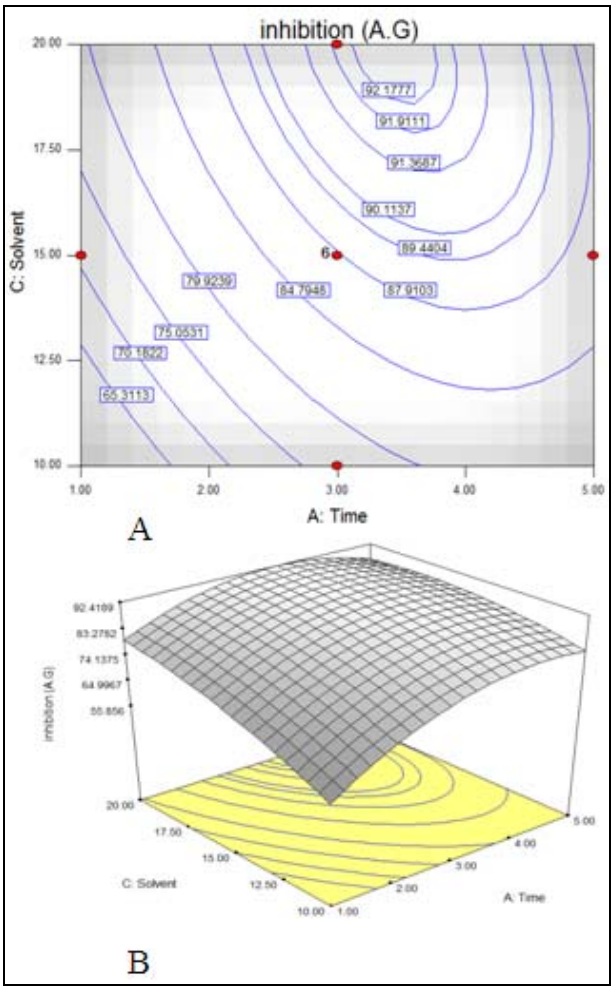

Fig. 3: (a) 2D contour plot and (b) 3D response surface show the effect of incubation time and amount of solvent, and their mutual interaction on percentage of α-glucosidase inhibition by *Piper cubeba.*

Optimization of P. cubeba L. (cubab) aqueous extract for the α-glucosidase inhibition activity

For the prediction of the optimal values of α-glucosidase inhibition activity yield within the experimental constrains, second-order polynomial equation was fixed to the mean data values to obtain regression equation. Data were fitted by the following quadratic polynomial equation below:

$$Y_1 = +87.77 +7.99A + 0.25B + 7.56C - 8.87A^2 -7.35B^2 - 3.31C^2 + 3.37AB - 4.18AC -0.31BC$$

Where, Yis the enzyme inhibition response, while A, B, C are the coded values for the variables of time, temperature and solvent volume, respectively. A positive value indicates it will have a positive effect, from the equations we can conclude that the higher the value the more inhibition percentage will be, whereas the negative sign shows an inverse relationship.α-glucosidase inhibitor activity reached the highest value in run 5 at incubation time = 3 days, temperature = 30 °C and volume of solvent extraction = 15 mL.

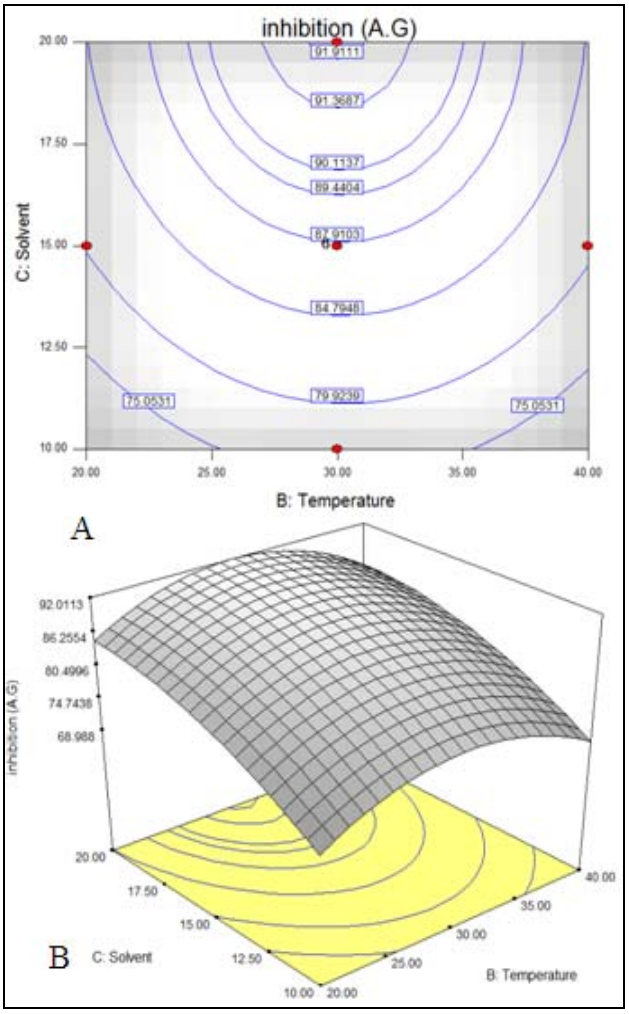

Fig. 4: (a) 2D contour plot and (b) 3D response surface show the effect of temperature and amount of solvent, and their mutual interaction on percentage of α-glucosidase inhibition by *Piper cubeba.*

Analysis using response surface methodology (RSM)

The contour plot and their corresponding three dimensional (3D) response surface for the α-glucosidase inhibition activity against any two independent variables at zero level are presented in figs. An elliptical response surface in the entire region was observed from the second order quadratic equation for the α-glucosidase inhibitor activity with the interaction of incubation time and temperature (fig. 2).

The results show that the activity of α-glucosidase inhibitor was considerably affected by varying the incubation time and temperature. About 89.67% inhibition activity was achieved from the response surface as the maximum inhibition activity at incubation time (4 days),

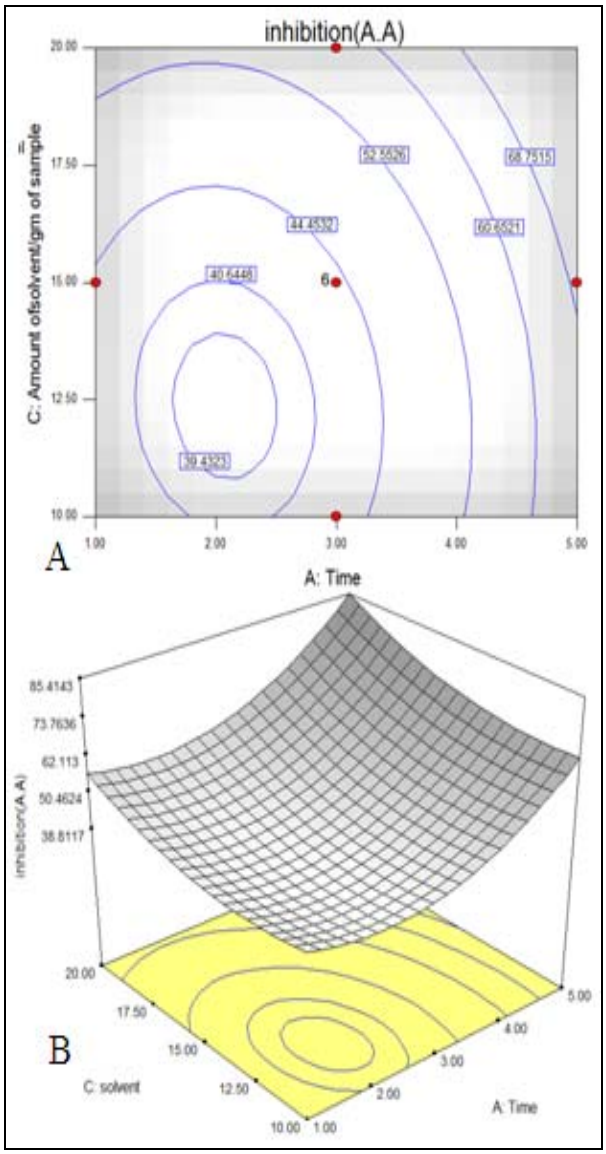

Fig. 5: (a) 2D contour plot and (b) 3D response surface show the effect of incubation time and amount of solvent, and their mutual interaction on percentage of α-amylase inhibition by *Piper cubeba.*

temperature (31 °C), and solvent (15 mL), respectively. Fig. 3 is the response surface plot for inhibitory activity of α-glucosidase, as a function of incubation time and amount of solvent by keeping the value of temperature at 30 °C. Maximum activity of α-glucosidase inhibitor (92.42%) was obtained when incubation time was about 4 days and solvent was about 18.5 mL. Fig. 4 also demonstrates the elliptical response surface plot of α-glucosidase inhibitor activity as a function of temperature and amount of solvent. The predicted inhibitor activity reduced at the higher and lower values of ranges for both temperature and solvent values. The maximum activity of α-glucosidase inhibition of about 92.01% was predicted at the temperature and amount of solvent of about 30 °C and 15 mL, respectively; while incubation time remained constant for 3 days.

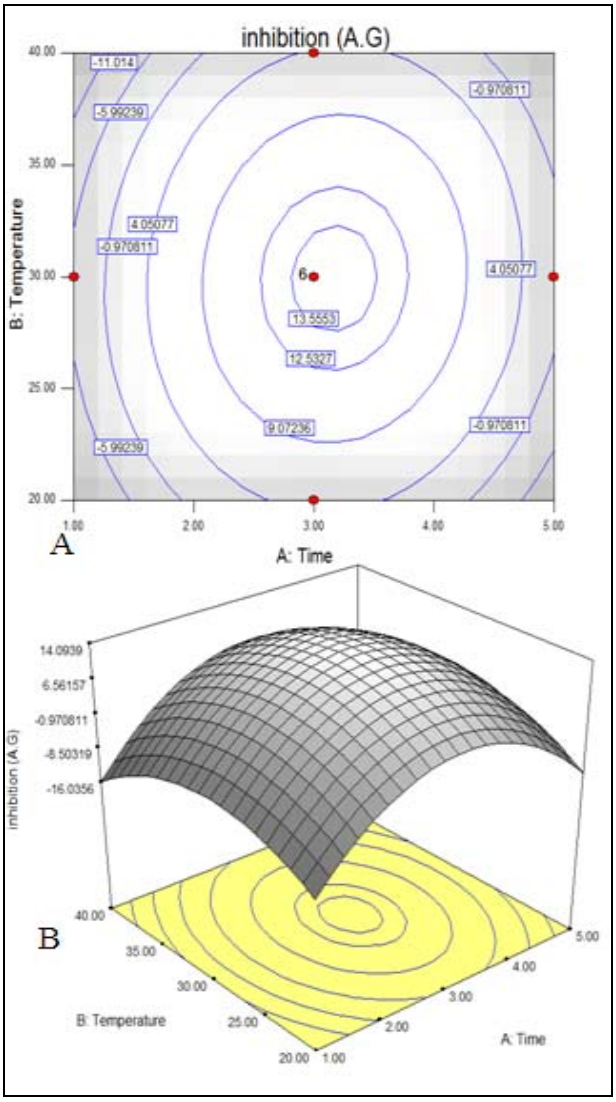

Fig. 6: (a) 2D contour plot and (b) 3D response surface show the effect of incubation time and temperature and their mutual interaction on percentage of α-glucosidase inhibition by *Elettaria cardamomum*.

Optimization of Piper cubeba L. (cubab)aqueousextract for theα-amylase inhibition activity

Data were fitted by the following quadratic polynomial equation below:

$Y2 = + 43.78 + 12.98A - 4.97B + 7.52C - 13.13A^2 - 9.37B^2 + 6.46\ C^2 - 11.20AB + 1.54AC + 10.46BC$

α-amylase inhibitor activity reached the highest value in run 14 at incubation time = 5 days, temperature = 20°C and volume of solvent extraction = 20mL.

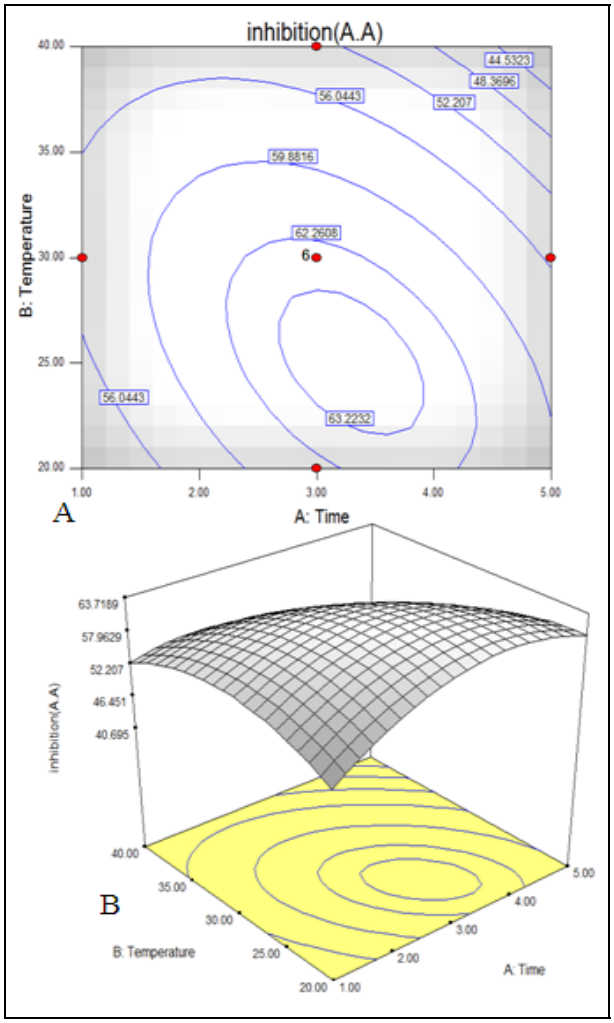

Fig. 7: (a) 2D contour plot and (b) 3D response surface show the effect of incubation time and temperature, and their mutual interaction on percentage of α-amylase inhibition by *Elettaria cardamomum.*

Analysis using response surface methodology (RSM)

The results shown in fig. 5, the activity of α-amylase inhibitor was considerably affected by varying the incubation time and amount of solvent by keeping the value of temperature at 30 °C. Maximum activity of α-amylase inhibitor (85.41%) was obtained when incubation time was about 2 days and solvent was about 11.5 mL. Interaction between incubation time with temperature and temperature with solvent shows no significant interrelation in the response surface plate.

Optimization of Elettaria cardamomum* L. *(cardamom*) *aqueousextract for theα-glucosidase inhibition activity

Data were fitted by the following quadratic polynomial equation below:

$Y3= + 13.97+ 2.83A - 0.25B – 5.56C – 16.38A^2 -9.31B^2 + 18.29C^2+ 1.24AB - 4.18AC -0.28BC$

α-glucosidase inhibitor activity reached the highest value in run 13 at incubation time = 3 days, temperature = 30 °C and volume of solvent extraction = 10 mL.

Analysis using response surface methodology (RSM)

An elliptical response surface in the entire region was observed from the 2nd order quadratic equation for the α-glucosidase inhibitor activity with the interaction of incubation time and temperature (fig. 6). The activity reduced at the minimum and maximum values of ranges considered in both parameters. Around 14.09% inhibition activity was achieved from the response surface as the maximum inhibition activity at incubation time (3 days). Interaction between incubation time with solvent amount and temperature with solvent shows no significant interrelation in the response surface plate.

Optimization of Elettaria cardamomum* L. (cardamom) *aqueous extract for the α-amylase inhibition activity

Data were fitted by the following equation quadratic polynomial equation below:

$Y4 = + 62.67 – 0.69A - 4.48B -0.51C – 6.37A^2 - 5.28B^2+ 5.26 C^2- 5.17AB – 2.06AC + 0.32BC$

α-amylase inhibitor activity reached the highest value in run 15 at incubation time = 5 days, temperature = 20 °C and volume of solvent extraction = 10 mL.

Analysis using response surface methodology (RSM)

The results shown in fig. 7 explain that the activity of α-amylase inhibitor was greatly affected by changing the incubation time and temperature as well as by keeping the value of solvent at 15mL. Maximum activity of α-amylase inhibitor (63.72%) was obtained when incubation time was about 3.5 days and temperature was about 25 °C. Interaction between incubation time with solvent and temperature with solvent shows no significant interrelation in the response surface plate.

The table 6 summarizes about the four optimization experiments, the values of higher R-Squared (more than 0.75) pointed out to a high significance of the model and indicated the appropriateness of the model (Alam *et al*., 2008; Khuri & Mukhopadhyay 2010). For both extracts, it was found that 3 days can give the maximum α-glucosidase inhibition. Also, for both extracts, 5 days can give the maximum α-amylase inhibition.

In our study, *P. cubeba* (cubab) aqueous extract for the α-glucosidase inhibition activity showed significant interrelation in response to surface plot for the interactions betweenincubation time and temperature, incubation time and amount of solvent, and temperature and amount of solvent, while α-amylase inhibition was considerably affected by varying the incubation time and amount of solvent. For *E. cardamomum* (cardamom), α-glucosidase and α-amylase inhibitions were considerably

affected by varying the incubation time and temperatures; therefore, these are the main variables that must be needed to be considered each time when studying the antidiabetic effect of *E. cardamomum*. We can conclude that all the optimization experiments were affected by incubation time. This was also supported by the study done by Tanyildizi *et al*. (2005) who reported that the incubation time can play a major role in affecting the results for the optimization experiments.

Combination of the extracts

The extracts were mixed with respect to find their effects whether they can improve the activity as compared to if they were given alone. The interaction may be antagonistic, synergistic or additive. It is needed to carry out a systematic evaluation of such permutations. From our study, it was found out that the best design was shown with cubab, and cubab mixed extracts, this indicates that cubab might have a synergistic effect when mixed with other extracts. The result also support that cubab may be a good plant to be used in treating DM, yet, further *in-vivo* investigations are needed to be carried out before drawing a final conclusion.

CONCLUSION

Diabetes mellitus is an assemblage of metabolic diseases strongly associated with chronic hyperglycemia resulting from deficiencies in insulin production in human body. Genetic factors and food habits have been strongly suggested to be responsible for the occurrence of DM. Hyperglycaemia in T2DM is a main infirmity, which is manifested by an abnormal post-prandial upsurge of the levels of blood glucose in human body. Several medicinal plants or natural products have been evaluated for their role to reduce glucose production from the digestive carbohydrates in the gut or glucose absorption from the intestine, through this way; they have been reported to reduce the post-prandial hyperglycaemia significantly. This research was aimed to study the antidiabetic activities of aqueous and methanol extracts of three traditional medicinal plants through different methods to evaluate their *in vitro* inhibitory properties against digestive enzymes i.e. α-amylase and α-glucosidase. Owing to the fact that antioxidants do exist in plants can play preventive role in the development of diabetes mellitus by decreasing the antioxidant level, therefore, measurement of antioxidant activity of the plants was also carried out through the estimation of DPPH, FRAP, total flavonoid and phenolic contents tests. Among the six crude extracts of three plants studied, aqueous extracts of *P. cubeba* and*E. cardamomum* showed a reasonable inhibitory property on both the enzymes with greater inhibitory activity was manifested on α-glucosidase in comparison to α-amylase inhibitory activity. They also showed good *in-vitro* antioxidant activities. Further optimization studies were performed with *P. cubeba* and*E. cardamomum* extracts. The optimization increased the *E. cardamomum*'s α-glucosidase inhibitory activity. The combination of the three aqueous extracts was performed and cubab was found to display the best and most consistent results. In conclusion, from our results, we can say that our study's results can further support the view of the fact that medicinal plants are potential sources of natural antidiabetic and antioxidants agents. The results of this study further justify the usage of these traditional medicinal herbs as dietary supplements in the prevention of diabetes, and their effective α-glucosidase and α-amylase inhibitory property is being confirmed and reported for the first time through this study. However, further studies are still required to find out active principles responsible and the mode of action of *P. cubeba* and*E. cardamomum* as digestive enzymes inhibitors with respect to manage diabetes efficaciously.

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REFERENCES

Adolfo A and Heinrich M (2005). Mexican plants with hypoglycaemic effect used in the treatment of diabetes. *J. Ethnopharmacol.,* **99**: 325-348.

Ahmad M, Qureshi R, Arshad M, Khan A and Zafar M (2009). Traditional herbal remedies used for the treatment of diabetes from district attock (Pakistan). *Pak. J. Bot.,* **41**: 2777-2782.

Ahmed QU, Dogarai BBS, Amiroudine MZAM, Taher M, Latip J, Umar A and Muhammad BY (2012). Antidiabetic activity of the leaves of *Tetracera indica* Merr. (Dilleniaceae) *in vivo* and *in vitro*. *J. Med. Plants Res.,* **6**: 5912-5922.

Alam MN, Bristi NJ and Rafiquzzaman M (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical J*., **21**: 143-152.

Alam MZ, Jamal P and Nadzir MM (2008). Bioconversion of palm oil mill effluent for citric acid production: Statistical optimization of fermentation media and time by central composite design. *World J. Microb. Biot.,* **24**: 1177-1185.

Al-Qirim TM, Shahwan M, Zaidi KR, Ahmed QU and Banu N (2002). Effect of Khat, its constituents and restraint stress on free radical metabolism of rats. *J. Ethnopharmacol.,* **83**: 245-250.

Apostolidis E, Kwon YII and Shetty K (2007). Inhibitory potential of herb, fruit, and fungal-enriched cheese against key enzymes linked to type 2 diabetes and

hypertension. *Innov. Food Sci. Emerg. Technol.,* **8**: 46-54.

Benzie I and Strain J (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration: *Methods Enzymol.,* **299**: 15-27.

Berger B, Stenström G and Sundkvist G (1999). Incidence, prevalence, and mortality of diabetes in a large population: a report from the Skaraborg diabetes registry. *Diabetes Care*, **22**:773-778.

Carrascosa JM, Molero JC, Fermin Y, Martinez C, Andres A and Satrustegui J (2001). Effects of chronic treatment with acarbose on glucose and lipid metabolism in obese diabetic Wistar rats. *Diab. Obes. Metab.*, **3**: 240-248.

Cheplick S, Kwon YI, Bhowmik P and Shetty K (2010). Phenolic linked variation in strawberry cultivars for potential dietary management of hyperglycemia and related complications of hypertension. *Bioresour. Technol.,* **101**: 404-413.

Cioanca O, Mircea C, Hritcu L, Trifan A, Mihasan M, Aprotosoaie AC, Robu S, Gille E and Hancianu M (2015). *In vitro - in vivo* correlation of the antioxidant capacity of *Salviae aetheroleum* essential oil. *Farmacia*., **63**: 34-39.

Djeridane A, Hamdi A, Bensania W, Cheifa K, Lakhdari I and Yousfi M (2015). The *in vitro* evaluation of antioxidative activity, α-glucosidase and α-amylase enzyme inhibitory of natural phenolic extracts. *Diabetes Metab. Syndr.,* **9**: 324-331.

Duke JA (1992). Handbook of Phytochemical Constituents of GRAS Herbs and other Economical Plants. London: CRC Press, pp.239-240.

Egwaikhide PA, Okeniy SO and Gimba CE (2009). Screening for antimicrobial activity and phytochemical. *J. Med. Plants Res.,* **3**: 1088-1091.

Esra K, Mustafa A, I lhan G and Erdem Y (2004). Evaluation of *in vivo* biological activity profile of isoorientin. Z. *Naturforsch.*, **59**: 787-790.

Gopalakrishnan M, Narayanan C and Grenz M (1990). Non saponifiable lipid constituents of cardamom. *J. Agric. Food. Chem.,* **38**: 2133-2136.

Herbal Medicine Research Centre (HMRC) (2002). Compendium of medicinal plants used in Malaysia (Vol. 2). Kuala Lumpur: Institute for Medical Research. pp.226-227.

Horii S, Fukase H, Matsuo T, Kameda Y, Asano N and Matsui K (1986). Synthesis and alpha-D glucosidase inhibitory activity of N-substituted valiolamine derivatives as potential oral antidiabetic agents. *J. Med. Chem.*, **29**: 1038-1046.

International Diabetes Federation, Diabetes Atlas, International Diabetes Federation, Brussels, Belgium, 3rd edition, 2006.

Jarald E, Joshi SB and Jain DC (2008). Diabetes and Herbal Medicines. *Iranian J. Pharmacol. Ther.,* **71**: 97-106.

Kaleem M, Baba SP, Ahmed QU and Bano B (2006). Antidiabetic and antioxidant activity of *Annona squamosa* extract in streptozotocin induced diabetic rats.*Singapore Med. J.,***47**: 670-675.

Kaleem M, Kirmani D, Asif M, Ahmed QU and Bano B (2006). Biochemical effects of *Nigella sativa* seeds in diabetic rats. *Indian J. Exp. Biol.,* **44**: 745-748.

Kaleem M, Medha P, Ahmed QU,Asif M and Bano B (2008). Beneficial effects of *Annona squamosa* extract in streptozotocin induced diabetic rats. *Singapore Med. J.,* **49**: 800-804.

Khan AV, Ahmed QU**,** Khan AA and Shukla I (2010). Antibacterial activity of *Bacopa monneiri* (L.) Wettst. (Scrophulariaceae) crude leaf extracts against some tropical human pathogenic bacteria. *Asian Biomed.,* **4**: 651-655.

Khuri AI and Mukhopadhyay S (2010). Response surface methodology." In: Wiley Interdisciplinary Reviews - Computational Statistics, Vol. 2 (2). E. J. Wegman, Y. H. Said, and D. W. Scott, Editors. Wiley, Hoboken, New Jersey, USA. pp. 128-149.

King H, Aubert RE and Herman WH (1998). Global burden of diabetes, 1995-2025: Prevalence, numerical estimates, and projections. *Diabetes Care*, **21**: 1414-1431.

Kuda T and IkemoriT (2009). Minerals, polysaccharides and antioxidant properties of aqueous solutions obtained from macro algal beach casts in the Noto Peninsula, Ishikawa. *Japanese Food. Chem.*, **112**: 575-581.

Marcia Da SP, Kwon YI, Apostolidis E, Franco ML, Maria IG and Shetty K (2008). Functionality of bioactive compounds in Brazilian strawberry (*Fragariax ananassa* Duch.) cultivars: Evaluation of hyperglycemia and hypertension potential using *in vitro* models. *J. Agric. Food. Chem.*, **56**: 4386-4392.

Masoko P, Picard J and Eloff JN (2005).Antifungal activities of six South African *Terminalia* species (Combretaceae). *J. Ethnopharmacol.,* **99**: 301-308.

McCue P, Kwon YII and Shetty K (2005). Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia Pac. J. Clin. Nutr.,* **14**: 145-152.

Nahak G and Sahu R (2011). Phytochemical evaluation and antioxidant activity of *Piper cubeba* and *Piper nigrum*. *J. Applied Pharmaceutical Science,* **1**: 153-157.

Nickavar B and Yousefian N (2011). Evaluation of α-amylase inhibitory activities of selected antidiabetic medicinal plants. *J. Verbrauch. Lebensm.*, **6**:191-195.

Okugawa H, Moriyasu M, Matsushita S, Saiki K, Hashimoto Y, Matsumoto K, Fujioka A and Kato A (1988). Evaluation of crude drugs by a combination of enfleurage and chromatography on flavor components

in seeds of *Amomum cardamomum* and *Elettaria cardamomum*. *Shoyakugaku Zasshi.,* **42**: 94-97.

Ordonez AAL, Gomez JD, Vattuone MA and Isla MI (2006). Antioxidant activity of *Sechium edule* (Jacq.)Swart extracts. *Food Chem*., **97**: 431-437.

Parasuraman S, Thing GS and Dhanaraj SA (2014).Polyherbal formulation: Concept of Ayurveda. *Pharmacogn. Rev*., **8**: 73-80.

Patthamakanokporn O, Puwastien P, Nitithamyong A and Sirichakwal PP (2008). Changes of antioxidant activity and total phenolic compounds during storages of selected fruit. *J. Food Comp. Anal.,* **21**: 241-248.

Perez-Jimenez P, Arranz S, Tabernero M, Diaz-Rubio ME, Serrano J, Goni I and Saura CF (2008). Updated methodology to determine antioxidant capacity in plant foods, oils and beverages: Extraction, measurement and expression of results. *Food Res. Intl.,* **41**: 274-285.

Shaban MA, Kandeel KM, Yacout GA and Mehaseb SE (1987). The chemical composition of the volatile oil of *Elettaria cardamomum* seeds. *Pharmazie.,* **42**: 207-208

Shaw JE, Sicree RA and Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res. Clin. Pract.*, **87**: 4-14.

Sima AAF and Chakrabarti S (2004). Long-term suppression of postprandial hyperglycaemia with acarbose retards the development of neuropathies in the BB/W-rat. *Diabetologia,* **35**: 325-330.

Sulaiman SF, Yaacob SS, Lan TM and Muhammad TS (2008). Chemical components of the essential oils from three species of Malaysian *plumeria* L. and their effects on the growth of selected microorganisms. *J. Biosci.,* **19**: 1-7.

Taher M, Amiroudine MZAM, Zakaria TMFST, Susanti D, Ichwan SJAI, Kaderi MA, Ahmed QU and Zakaria ZA (2015). Alpha-mangostin improves glucose uptake and inhibits adipocytes differentiation in 3T3-L1 cells via PPAR, GLUT4 and Leptinexpressions. *Evidence-Based Comp. Alt. Med.,* ID 740238.

Tanyildizi MS, Ozer D and Elibol M (2005). Optimization of α-amylase production by *Bacillus sp*. using response surface methodology. *Process Biochem.,* **40**: 2291-2296.

Tunna TS, Ahmed QU, Sarker ZI, Ghafoor K, Al-Juhaimi FY, Uddin S, Hasan M and Ferdous S (2015). Analyses and profiling of extract and fractions of neglected weed *Mimosa pudica* Linn. traditionally used in Southeast Asia to treat diabetes. *South African J. Bot.*, **99**: 144-152.

Umar A, Ahmed QU*,* Muhammad BY, Dogarai BBS and Mat Soad SZ (2010). Anti-hyperglycemic activity of the leaves of *Tetracera scandens* Linn.Merr. (Dilleniaceae) in alloxan induced diabetic rats. *J. Ethnopharmacol.,* **131**: 140-145.

Wang Y, Huang S, Shao S, Qian L and Xu P (2012). Studies on bioactivities of tea (*Camellia sinensis* L.) fruit peel extracts: Antioxidant activity and inhibitory potential against alpha-glucosidase and a-amylase *in vitro*. *Industrial Crop Prod.*, **37**: 520-526.

World Health Organization(WHO) (2012). Diabetes mellitus. Retrieved February 7, 2012 from http://www.who.int/topics/diabetes_mellitus/en/

Xie Z, Yunan Z, Dayong G, Lijun D, Guoping C and Yaou Z (2011). Scorpion in combination with gypsum: Novel antidiabetic activities in streptozotocin-induced diabetic mice by up-regulating pancreatic PPARg and PDX-1 expressions. *Evidence-Based Comp. Alt. Med.,* pp.1-9.

Yankuzo H, Ahmed QU, Santosa RM, Akter SFU and Talib NA (2011). Beneficial effect of the leaves of *M. koenigii* (L.) Spreng (Rutaceae) on diabetes induced renal damage *in vivo*. *J. Ethnopharmacol.,* **135**: 88-94.

Zhang P, Zhang X, Brown J, Vistisenc D, Sicree R, Shaw J and Nichols G (2010). Global healthcare expenditure on diabetes for 2010 and 2030. *Diabetes Res. Clin. Pract.*, **87**: 293-301.

Zheng W and Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. *J. Agric*. *Food Chem.,* **49**: 5165-5170.