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Research Article

Evaluation of *in vitro* cholesterol esterase and α-amylase inhibitory activities of purified polyphenols from *Nigella sativa* seeds

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ABSTRACT

α-amylase hydrolyses starch into maltose, and cholesterol esterase hydrolyzes cholesterol ester into cholesterol and fatty acids in the lumen of small intestine. Regulated inhibition of the above cited enzymes can effectively control diabetes mellitus and associated cardiovascular diseases. In the above cited context, *Nigella sativa* (Black cumin) seeds were investigated for *in vitro* amylase and cholesterol esterase inhibitory properties. The amylase and cholesterol esterase inhibitory activities of hot water extract [HWE] (96.4±1.5% & 90±2.5%, respectively) and organic solvent extract [OSE] (98.1±0.5% & 96.6±1.2%, respectively) were found to be satisfactory. Appreciable amount of total phenolic content (TPC) and total flavonoid content (TFC) was observed in both the extracts. The thin layer chromatography (TLC) analysis of HWE has recorded an R_f value of 0.94 which established the presence of polyphenols. The purified polyphenol eluate by 3D preparative thin layer chromatography (PTLC) has recorded a moderate α-amylase (46.7±3.2%) and cholesterol esterase (32.6±1.7%) inhibitory activities. The polyphenols coated with cotton fabric, and stainless steel implant possessed noteworthy α-amylase and cholesterol esterase inhibitory activities has revealed the promisi ng use of *Nigella sativa* in the healthcare industry to treat diabetes mellitus and cardiovascular diseases.

Keywords: α-Amylase; Cholesterol esterase; Diabetes mellitus; *Nigella sativa*; Polyphenol.

INTRODUCTION

Diabetes Mellitus is a major chronic endocrine/ metabolic disorder caused due to inherited acquired deficiency of the β-cells of pancreas to produce insulin or loss of insulin sensitization by insulin receptors or by both. Diabetes mellitus is the most challenging societal oriented health problem that had a worldwide prevalence that have affected about 387 million (8.3%) people and predicted to rose up to 592 million by 2035 (Gyawali B *et al.,* 2015). India is considered as the "Diabetes capital of the world" since; it tops the world with the highest number of diabetic patients (Mohan V *et al.,* 2007). In India, more than 62 million individuals were diagnosed with diabetes mellitus and the status seemed to be rapidly increasing day by day. The preliminary results published by Indian council of medica l research (ICMR) has revealed that only a minor population of northern India (Jharkhand 0.96 million and Chandigarh 0.12 million) were affected with diabetes compared with the southern parts (Tamil Nadu 4.8 million and Maharashtra 9.2 million) (Kaveeshwar SA and Cornwall J, 2014). The best strategy to manage diabetes mellitus is to regulate blood glucose level through the inhibition of few key regulatory enzymes. In this connection, drugs that effectively inhibit α-amylase play a major role in the decrease of postprandial hyperglycemia and thereby, diabetes mellitus. But, due to their severe gastrointestinal adverse effects, current pharmaceutical market has focused plant extracts or plant based purified products which are considered to be efficacy, non-toxic, posing minimal or nil side effects to regulate diabetes mellitus (Poongunran J *et al.,* 2015)

Cardiovascular diseases (CVD) such as myocardial infarction, ischemic heart disease and heat attack are the prime cause for severe mortality rate in many parts of the World and responsible for more than 40% of annual death. It has been observed that about 80% people belongs to low and middle income countries were affected by CVD based mortality and the numerical is expected to grow up to 23.6 million by 2030 (Reddy KS, 2004). In India, about 7 to 13% urban, and 2 to7% rural population were estimated to be affected by CVD. Poorly managed CVD leads to significant long term complications such as heart attack, stroke, heart failure and renal diseases. Heart attack and stroke are

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acute events caused due to the risk factors such as hypertension, diabetes mellitus, hyperlipidemia, atherosclerosis, usage of tobacco, unhealthy diet and obesity. Increased levels of triglycerides, fatty acids and cholesterol have a strong correlation in the development of several CVDs. Hence, inhibition of enzymes such as lipase and cholesterol esterase by the drugs has proved to prevent the hydrolysis of lipids and cholesterol esters, and thereby, an effective control over CVDs (Adisakwattana S *et al.,* 2012).

Nigella sativa is one of the major medicinal plant spices that have been used since ancient times to cure many human ailments. It is most commonly known as the Black Cumin seeds and possesses special healing properties (Naz H, 2011). In India, the seeds are used i n culinary because of it's strong pepper taste and also used as a nutritional supplement (Ramadan MF and Mörsel JT, 2004). It has been reported that the seeds have many properties such as diuretic, antibacterial, anti-tumour, anti-inflammatory, antihypertensive, antidiabetic, anticancer, immunomodulatory, analgesic, anthelmintics, spasmolytic, bronchodilator, hepato, reno and gastro protective, and antioxidant (Ahmad A *et al.,* 2013; Ghosheh OA *et al.,* 1999; Houghton PJ *et al.,* 1995; Morsi NM, 2000).

To ensure our security and safety from the health oriented hazards, we need to equally develop the technology for our protection. With regard to textiles, the protective textile field of the smart textiles can fulfill this requirement. All over the world there i s huge demand on global scale for the functionality of the textile products like wrinkle resistance, water repelling, fade resistance and resistance to microbial invasion, and other value added medicinal properties. The consumers are aware of hygienic life style and there is a necessity of textile product with antimicrobial, antidiabetic and antihypercholesterolemic properties with the use of herbs and herbal products.

Based upon the above cited scientific documentation reviewed, the present work done in our laboratory has aimed the screening of α-amylase and cholesterol esterase inhibitory property from the seeds of *Nigella sativa* and also the probable role of polyphenols in the above mentioned activity. Attempts have been also made to develop value added cotton fabric and simulated implant models to study the inhibitory property of the quercetin and purified polyphenols.

EXPERIMENTAL

Preparation of Sample

Black Cumin seeds (*Nigella sativa*) were purchased from the local market at Coimbatore, Tamil Nadu, India during the period between July to September 2015. The seeds were homogenized with the help of mechanical homogenizer and were sieved manually to obtain fine powder. The powder was used for further experimental analysis.

Preparation of Hot Water Extract (HWE)

1 g of sample was weighed and extracted with 25 ml of distilled water in the water bath at 90°C (Deve AS *et al.,* 2014). The extract was filtered using Whatman No.1 filter paper, the filtrate obtained was then precipitated with 80% ammonium sulphate and centrifuged at 10,000 rpm for 10 minutes. The obtained supernatant was used for further analysis.

Preparation of organic solvent extract (OSE) by shake flask method

2.5 g of sample was weighed and added to 50 ml of various organic solvents like petroleum ether, chloroform, acetone, ethyl acetate, ethanol, methanol and distilled water each separately, and shook overnight at 100 rpm and was filtered using Whatman No.1 filter paper. The filtrate obtained was precipitated with 80% ammonium sulphate and centrifuged at 10,000 rpm for 10 minutes, and the obtained supernatant was used for further analysis. 1 ml of each solvent extract was mixed to obtain a common pooled fraction (Sathishkumar T and Baskar R, 2014).

Preparation of OSE by Separating Funnel Method

In a clean dry 250 ml separating funnel, 5 g of the sample was taken and 25 ml of petroleum ether was added and shook vigorously for 15 minutes. The resultant mixture was filtered using Whatman No.1 filter paper and the residue was re-extracted with the same solvent. The procedure was repeated for the organic solvents such as chloroform, acetone, ethyl acetate, ethanol, methanol and distilled water each separately. The filtrates obtained were used for further analysis. 1ml of each sample solution was taken and mixed to obtain a common pooled fraction (Ghasemzadeh A and Ghasemzadeh N, 2011).

In vitro α-amylase inhibitory assay

Pipetted out 0.1ml of the sample in the “Blank” and “Test” tubes, and 0.1ml of amylase enzyme in the “Test” and “Control” tubes, respectively. Added 1ml of 50 mM phosphate buffer (pH 7.0) and 0.5ml of 1% starch into all the tubes and incubated at 37°C for 10 minutes. Added 1ml of 3, 5-dinitorsalicylic acid into all the tubes, mixed well and incubated in a boiling water bath for 10 minutes. Cooled the tubes and read the absorbance at 540nm against blank (Ashok L *et al.,* 2010). The inhibitory activity (%) was calculated as per the following formula: [(Activity of “C” - activity of “T”)/ activity of “C”] × 100.

In vitro cholesterol esterase inhibitory assay

A slightly modified method proposed by Kumar P *et al.,* (2011) was adopted for cholesterol esterase inhibitory assay. In a clean test tube, 2ml of p-nitrophenyl acetate (PNPA) and 1 ml of phosphate buffer pH 7.0 was added. Then, 0.5 ml of detergent (sodium taurocholate) and 0.5 ml of the sample was added, and the tube was mixed well. Added 0.1 ml of cholesterol esterase and

incubated at room temperature for 5 minutes. The "control" tube was prepared without adding the test sample. The absorbance of the formed yellow color was measured spectrophotometrically at 410 nm. The inhibitory activity (%) was calculated as per the following formula: [(Activity of "C" - activity of "T")/ activity of "C"] × 100.

Estimation of Total Phenolic Content (TPC) by Folin-Ciocalteau method

To 0.5 ml of the sample, added distilled water to make up the volume to 6 ml. To this, 0.5 ml of Folin-Ciocalteau reagent was added to all the test tubes and was incubated at room temperature for 3 minutes. 2 ml of 7% sodium carbonate was added and kept in a boiling water bath for one minute. The blue color formed was read spectrophotometrically against a blank at 650 nm (Singleton VL and Rossi JA, 1965). Gallic acid was used to construct the calibration curve.

Estimation of Total Flavonoid Content (TFC) by aluminium chloride method

About 0.5 ml of the sample, added distilled water to make up the volume to 4 ml. To this, 0.3 ml of 5% $NaNO_2$ and 0.3 ml of 10% $AlCl_3$ were added. After 6 minutes of incubation at room temperature, 2 ml of 1M NaOH and 3.4 ml of distilled water was added. The yellow color formed was read spectrophotometrically against a blank at 510 nm (Zhishen J *et al.,* 1999). Quercetin was used to construct the calibration curve.

Thin layer chromatography (2D TLC) and Preparative thin layer chromatography (3D PTLC)

Glass plate (20 × 20 cm) was coated (0.25 mm) with silica gel (40 g/80 ml water) as stationary phase. It was activated at 100°C for 30 minutes and kept at room temperature. 100µl of test sample was spotted from 2 cm above the base of the plate and one dimensional thin layer chromatogram was developed using the mobile phase composed of ethyl acetate: formic acid: acetic acid: distilled water (100: 11: 11: 26). Developed plates were air dried and visualized under long UV light (365nm). 2D TLC was carried out with the mobile phase composed of hexane: acetone (1:1) and the developed chromatogram was visualized under long UV light (365nm) (Sathishkumar T and Baskar R, 2014).

The PTLC technique was executed as per TLC analysis cited above with an incorporation of an additional mobile phase (methanol: distilled water (1:1)) as third dimension (3D) to purify the polyphenols. A strong single spot identified under long UV light (365nm) was carefully eluated and dissolved in acetate buffer (pH 5.5). The content was centrifuged at 10,000 rpm for 10 minutes and the supernatant collected was lyophilized, and used for further analysis.

Herbal Finish Application on Cotton by Exhaust Method

The HWE of *Nigella sativa* and purified polyphenols were applied onto the separate cotton fabric with the count of 2×50's with plain weave structure by exhaust method and Marybalon as a cross-linking agent at a concentration of 200 µg/ml with M:L of 1:5. The cotton fabric was immersed in the solution for about 45 minutes at 90^0C. It was then padded between the rollers for wet pickup. The fabric was then dried at 80°C for 10 min to remove the moisture and then cured for further enzyme inhibition analysis by immersing it in a simulated physiological condition (Ganesan P et al., 2013).

Square Thread Cutting

A SS316L stainless steel cylindrical solid rod of dimension 10 mm diameter and 50 mm length that represented a simulated implant with "V" profile cavities/ grooves to a length of 20 mm was prepared with the aid of a central lathe machine. The angle of the cavity was 60˚and the depth was 2 mm with a pitch of about 3.3 mm. Seven such cavities were constructed for loading quercetin. Sodium alginate (polymer) and quercetin (standard polyphenol) were taken in the ratio 4:1 and mixed with 25ml of distilled water to attain a semi-solid consistency. The mixture was coated in the grooves of the implant and dried. The physiological condition was simulated in terms of 0.02M phosphate buffer (pH 7) at 37̊ C and the implant was introduced into the simulated conditions for the investigation of enzyme inhibitory studies.

RESULTS AND DISCUSSION

***In vitro* α-amylase and cholesterol esterase inhibitory activity of different extraction methods**

In vitro α-amylase and cholesterol esterase inhibitory activity of *Nigella sativa* was studied by using different extraction methods and the results were depicted in the Fig. 1 and Fig. 2, respectively. The results proved that the hot water extract, shake flask method (distilled water) and separating funnel method (distilled water) possessed a maximum α-amylase inhibition of 96.4±1.5%, 98.1±0.5% and 96.7±0.8%, respectively. Similarly, maximum cholesterol esterase inhibitory activity was recorded for the hot water extract (90±2.5%), shake flask method (distilled water, 96.6±1.2% and separating funnel method (distilled water, 94.9±0.7%).

Organic solvent mediated extraction was carried out by both shake flask and separating funnel methods to determine the enzyme inhibitory activities. The solvents such as distilled water (Sh. Fl: 98.1±0.5%; Sep. Fun: 96.7±0.8%), ethanol (Sh. Fl: 92.8±1.1%; Sep. Fun: 85.6±2.5%), ethyl acetate (Sh. Fl: 95.8±0.3%; Sep. Fun: 92.9±0.5%) and chloroform (Sh. Fl: 94.3±1.5%; Sep. Fun: 90.9±0.3%) has recorded significant amylase inhibitory activity. Whereas, only distilled water (Sh. Fl: 96.6±1.2; Sep. Fun: 94.9±0.7%) and petroleum ether (Sh. Fl: 66.4±3.3%; Sep. Fun: 93.4±0.4%) has observed to possess high cholesterol esterase inhibitory activity.

Table 1: Phenolic and flavonoid contents of different extraction methods

Extraction methods	Solvents used	Phenolic Content (mg/g tissue)*	Flavonoid Content (mg/g tissue)*
Hot Water Extract	Distilled water	0.44±0.042	0.0058±0.001
Shake Flask Method	Petroleum Ether	0.055±0.011	0.143±0.044
	Chloroform	0.17±0.005	0.046±0.015
	Acetone	0.074±0.013	0.063±0.012
	Ethyl Acetate	0.203±0.006	0.058±0.052
	Ethanol	1.18±0.045	0.195±0.015
	Methanol	1.55±0.105	0.031±0.017
	Distilled water	1.05±0.098	0.044±0.015
	Pooled fraction	0.89±0.019	0.057±0.056
Separating Funnel Method	Petroleum Ether	0.09±0.006	0.017±0.008
	Chloroform	0.55±0.013	0.01±0.006
	Acetone	0.24±0.056	0.08±0.031
	Ethyl Acetate	0.17±0.017	0.005±0.002
	Ethanol	0.91±0.081	0.026±0.02
	Methanol	0.44±0.087	0.01±0.008
	Distilled water	0.082±0.014	0.026±0.011
	Pooled fraction	0.079±0.014	0.033±0.018

*Results were expressed as mean ± s.d

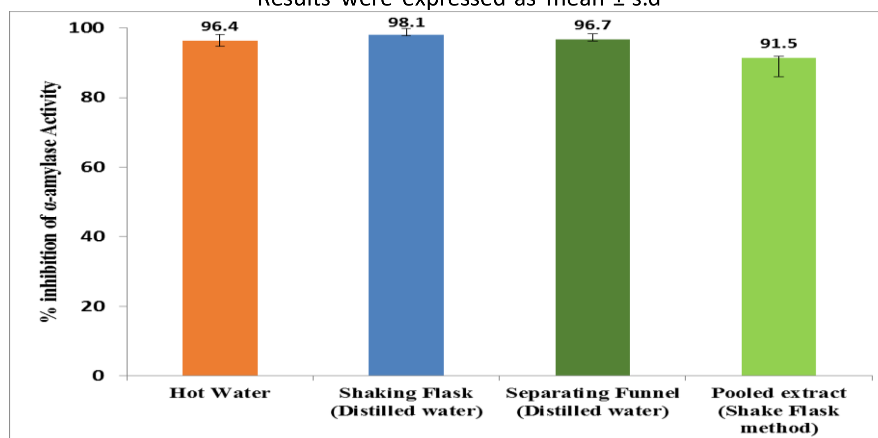


Figure 1: Comparison of α-amylase inhibitory activities of various extraction methods

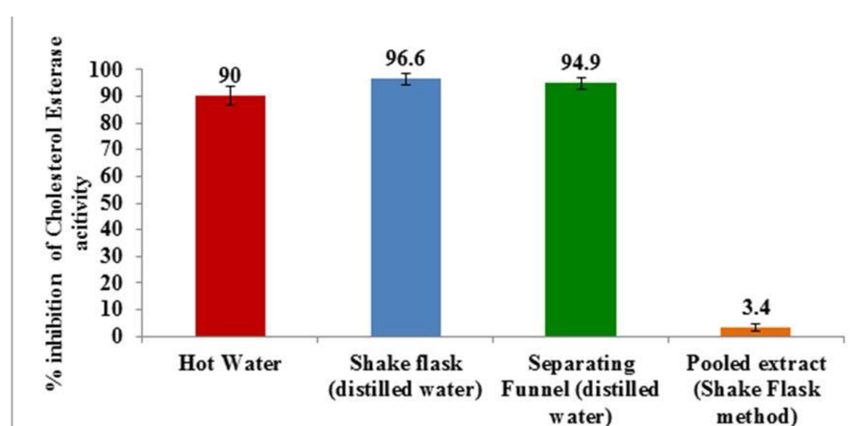
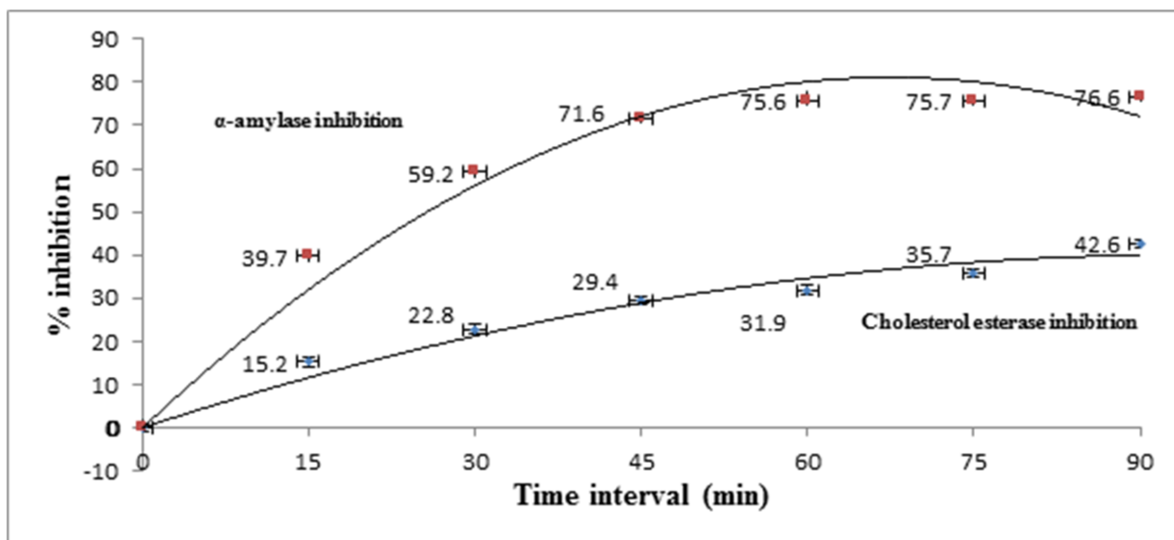


Figure 2: Comparison of cholesterol esterase inhibitory activities of various extraction methods

The results have revealed the potency of shake flask method in recording the highest α-amylase and cholesterol esterase inhibitory activities. But, hot water extract was selected for further analysis because of its non-toxicity, cost effectiveness, high dipole moment and less time consumption (Plaza M and Turner C, 2015). The increased inhibitory activity was due to the presence of the several phytoconstituents like alkaloids, polyphenols, sterols, tannins etc., from the seeds of *Nigella sativa*. Rabey HAE et al., (2017) has demonstrated a drastic reduction in the fasting serum glucose levels (significant difference at 0.1% level) in the streptozotocin (STZ) induced diabetic rats by the infusion of methanolic extract of *N. sativa* and the probable cause

Figure 3: 2D Thin layer chromatogram of HWE of N. sativa visualized under long UV light



(a)

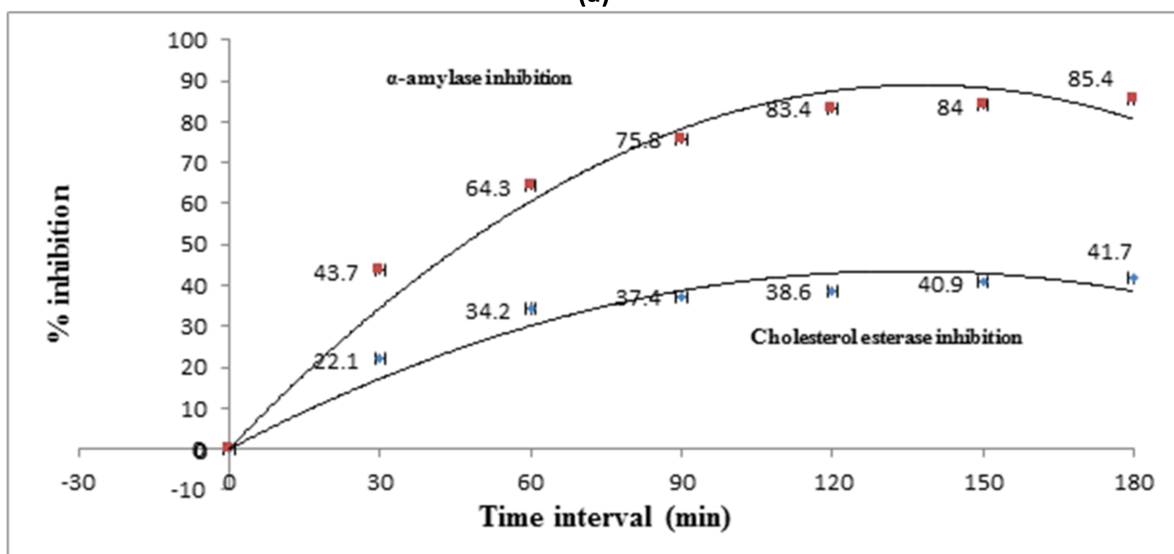


Figure 4: Controlled inhibition of α-amylase and cholesterol esterase by: (a) purified polyphenols; (b): quercetin

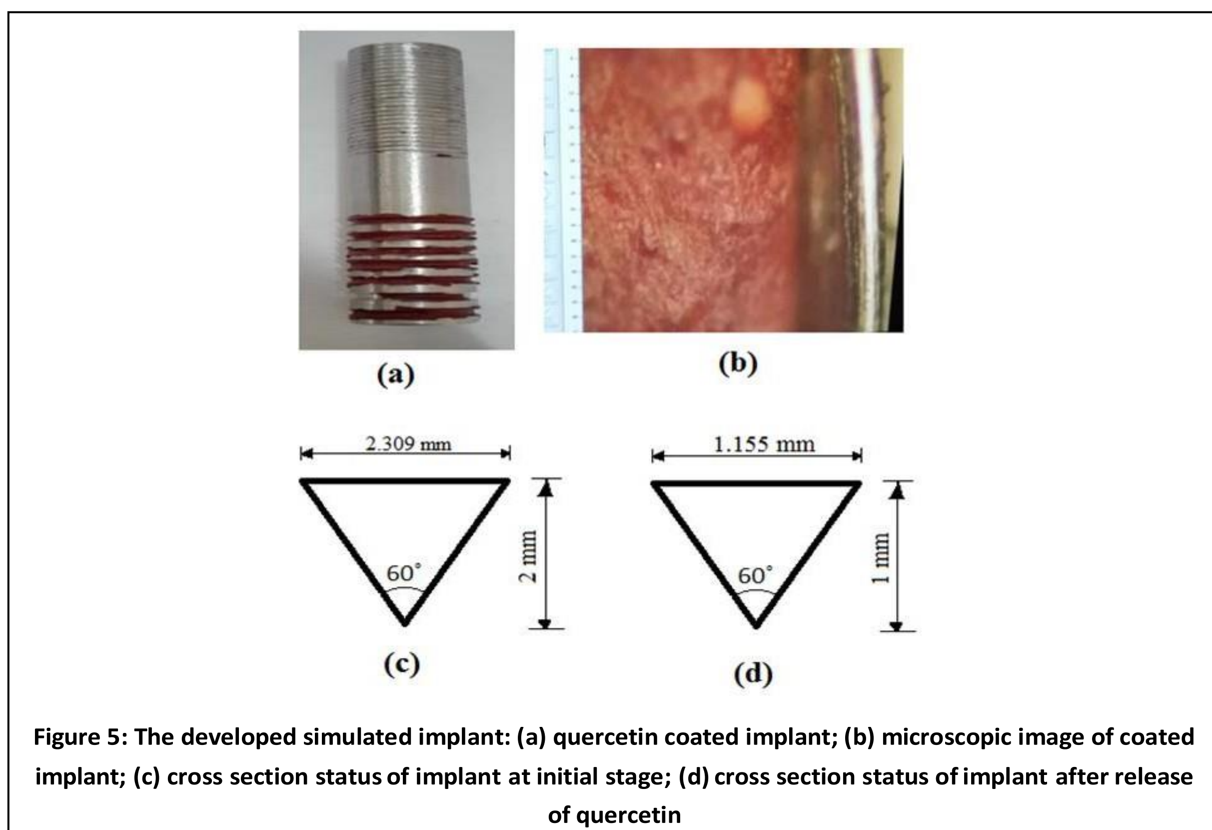


Figure 5: The developed simulated implant: (a) quercetin coated implant; (b) microscopic image of coated implant; (c) cross section status of implant at initial stage; (d) cross section status of implant after release of quercetin

for the decrease has been hypothesized due to the significant activity of phenolics and flavonoid content. The shake flask based aqueous leaf extract of *N. sativa* has recorded a significant amylase inhibitory activity (84%) (Sathiavelu A *et al.,* 2013). Very low (29%) and moderate (68%) amylase inhibitory activities were recorded by the ethyl acetate and methanol seed extract of *N. sativa*, respectively, (Amutha K and Godavari A, 2015; Belal H *et al.,* 2016) which is inferior than our current investigation results. The probable reason for the previously recorded low inhibitory activity was due to the soxhlet mode of phytochemical extraction which could have led a significant loss in the recovery of secondary metabolites and in the current study, a better extraction technique has been proposed. Likewise, a low pancreatic lipase inhibitory activity of methanolic seed extract of *N. sativa* has been recorded (Gholamhoseinian A *et al.,* 2010). *In vivo* oral treatment of *N. sativa* seeds to the Wistar rats have been proved to increase the HDL level, and a drastic decrease in LDL, total cholesterol and triglyceride levels (Paarakh PM, 2010). The supernatant obtained from the macerated seed powder suspended in phosphate buffer saline was found to be effective in inhibiting the porcine pancreatic lipase (Chedda U *et al.,* 2016). All the documented results strongly support our present investigation of amylase and cholesterol esterase inhibitory property of *N. sativa* seeds.

Total phenolic and flavonoid content present in *N. sativa*

An appreciable phenolic and flavonoid content was observed in the HWE, chloroform, ethanol, ethyl acetate and methanol extracts. Previous reports about the presence of polyphenols and flavonoid in the ethyl acetate, n-butanol and aqueous seed extracts have strongly supported the current investigation connected with the distribution of the above cited phytochemicals (Amutha K and Godavari A, 2015; Sheriff MS *et al.,* 2015). Similarly, a report documented by Rabey HAE *et al.,* (2017) has revealed about 9.9 mg/ g tissue of flavonoid content which is contradictory to our present result. Current study has recorded a maximum of 0.195 mg/ g tissue of flavonoid content. The lowest yield was probably due to the minimal time duration provided for the shake flask method to extract the phytocontents. The phenolic and flavonoid content of various extracts have been depicted in table 1.

Identification and isolation of polyphenols of *Nigella sativa* by 2D TLC and 3D PTLC techniques

The polyphenols present in the HWE were identified by 1D and 2D TLC techniques, respectively and were visualized under far UV light (360 nm). The observation of blue and dull yellow color spots revealed the presence of flavonoid. The recorded R_f values, 0.94 (Blue spot) and 0.83 (dull yellow spot) were well agreed with the previous recorded documentations (Kamal A and Ahmad IZ, 2014; Deve AS *et al.,* 2014). The 2D thin layer chromatogram was depicted in Fig 3.

PTLC is a significant method of sample purification for preparative purposes (10-1000 mg) and the eluate can be subjected for structural elucidation techniques such as mass spectrometry, IR spectroscopy, NMR etc., (Lade BD *et al.,* 2014). In the present study, a single strong spot of polyphenolic fraction was purified by 3D

PTLC technique. The purified eluate moderately inhibited both the α-amylase (46.7±3.2%) and cholesterol esterase (32.6±1.7%) which revealed the active participation of polyphenols in the inhibitory property.

Starch hydrolysis is the major route for the formation of postprandial blood glucose. Normally, α (1→4) glycosidic bonds of starch is hydrolyzed by salivary and pancreatic α-amylases to form maltose and oligosaccharides, which then, subsequently hydrolyzed to glucose by the α-glucosidases present in the brush border of small intestine cells (Williamson G, 2013). Hence, inhibition of enzymes such as amylase and glucosidase could be of novel strategy that can be adopted for an effective regulation of blood glucose. Even though, drugs such as acarbose is available in the market, plant based polyphenols can be recruited for the inhibition of the above mentioned enzymes due to their non-toxicity and minimal/ nil side effects (Hanhineva K *et al.,* 2010). In the previous documentation, researchers have proved the competitive inhibitory property of luteolin and quercetin against human salivary amylase (Nyambe-Silavwe H *et al.,* 2015). It has been proposed that the number of hydroxyl groups and presence of unsaturated 2, 3-bond in conjugation with a 4-carbonyl group may increase the inhibitory rate. The presence of sugar, methyl and methoxy groups can drastically decrease the inhibitory property (Xiao J *et al.,* 2013). Therefore, our current results related with the amylase inhibitory activity of purified polyphenolic content was well corroborated with the above cited reports.

Lipid digestion is mainly mediated by lipases, especially pancreatic lipase. Hydrolysis of lipids by lipase yields the end products such as free fatty acids, mono- and diacylglycerol, and glycerol. Similarly, digestion of cholesterol esters is mediated by pancreatic cholesterol esterase that leads to the formation of free cholesterol and fatty acids. Finally, the formed products are absorbed by the intestinal villi and an increased level of the above mentioned products may lead to various diseases such as hyperlipidemia, hypercholesterolemia and obesity. This subsequently progressed into many micro and macrovascular complications including angiopathy, angina pectoris, myocardial infarction, and cardiac arrest (Adisakwattana S *et al.,* 2012; Ado MA *et al.,* 2013). Previous reports of few plant species including *Nigella sativa* have well documented an appreciable lipase inhibitory activity. The inhibition kinetics has proved to be non-competitive predominantly than the other types (Ado MA *et al.,* 2013; Chedda U *et al.,* 2016; Gholamhoseinian A *et al.,* 2010). Recently, Shamsiya TK *et al.,* (2016) have reported a strong lipase inhibitory activity (92%) of mono- and polyunsaturated fatty acids purified from the ethanol extract of *N. Sativa* seed. Likewise, documentation by Bamosa AO *et al.,* (1997) has revealed a significant reduction in the total cholesterol and triglyceride levels in orally fed experimental rats by the *N. sativa* seeds, which was probably suggested due the action of thymoquinone. But, there are no reports about the cholesterol esterase inhibitory property of extracts and purified polyphenols of *N. sativa,* and it is first kind of scientific documentation recorded by our laboratory about the cited inhibitory property.

Fabrication by Exhaust Method

The inhibition of α-amylase and cholesterol esterase by quercetin and purified polyphenols coated onto a plain cotton fabric (separately) were studied and the results were depicted Fig. 4. Much regulated inhibition of cholesterol esterase (2% inhibition on average for every 30 minutes) by the quercetin and purified polyphenols was noticed than the inhibition of α-amylase (10% inhibition on average for every 30 minutes). After three hours, steady state kinetics in the release of quercetin and purified polyphenols was observed which, correlated with a regulated enzyme inhibition. This proved the significance of developed fabric as a valuable bandage for diabetic patients. Again, our laboratory is first to record a report on controlled enzyme inhibition by quercetin and purified polyphenols coated onto a fabric material.

Development of quercetin coated Implant Device

The quercetin coated in the SS316L stainless steel rod has been depicted in the Fig. 5a &b. Quercetin coated implant was found to inhibit the α-amylase in a rapid manner than the inhibition of cholesterol esterase. At the initial stage, the volume of the quercetin exposed in the simulated implant was about 440.16 mm^3, where the groove height was 2 mm and the corresponding area of the surface exposed was about 2538.88 mm^2 (Fig 5c). As the time progressed, quercetin was released only to about 1 mm height from the bottom of the groove, and hence, the volume of the quercetin exposed was about 93.1 mm^3 and the corresponding area of the surface exposed was 1244.59 mm^2 (Fig 5d).

The extent and rate at which the active moieties such as drug or metabolites that enter systemic circulation and then access the site of action is termed as bioavailability. Oral drug delivery is one of the easiest and simplest ways of administering drugs, but possesses less bioavailability and more drug load. Orally administered drugs pass through the intestinal wall and then into the portal circulation to the liver; which are common sites of first-pass metabolism and many drugs get metabolized in the systemic circulation itself before reaching the specific site. Most of these oral drugs are non-polar and are not well absorbed detracting the inherent efficacy of the drug (Vasconcelos T *et al.,* 2007). Thus, when the drug materials are coated in implants of specific site, it released from the implant slowly and efficiently. Thereby, an increased bioavailability and reduced drug load may be achieved in a cost effective manner (Regar E *et al.,* 2001). Our current investigation has proved a controlled release of quercetin from the implant but, still the release has to be regulated in or-

der to extend the time duration and also to reduce the cost.

CONCLUSION

The HWE of *Nigella sativa* seeds showed significant inhibition of α-amylase and cholesterol esterase. The TLC analysis revealed the presence of polyphenols and the purified polyphenols possessed moderate enzyme inhibitory activity. Similarly, the developed value added fabric (coated with quercetin and purified polyphenols), and the implant (coated with quercetin) were found to be effective in a regulated release of the above cited molecules and thereby, an effective inhibition of α-amylase and cholesterol esterase. In future, studies related to the structural prediction of the polyphenols and refinement of implant material can be attempted in order to have an effective control over diabetes mellitus and associated cardiovascular diseases.

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