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Anti-Oxidant and digestive enzymes inhibitory based anti diabetic activity of crude and fractions of *Carum carvi* L. extracts

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Abstract: In the present study crude ethanolic extract and its various fractions (ethyl acetate, hexane and aqueous) of medicinal plant *Carum carvi* L. were examined for α-amylase and α-glucosidase inhibition using an-in vitro model. Both digestive enzymes were extracted from bovine and green gram. The crude extract and its fractions were also studied for their antioxidant potential by DPPH and Nitric oxide activity. The quantitative assessment of phenol and flavonoid contents was also estimated. The crude extract and its fractions exhibited high in-vitro enzyme inhibitory activity against α-amylase and α-glucosidase at different concentrations with IC_{50} ranging from 421.4±7.8 to 810±5.71and 72±8.81 to 307.0±11.42µg/mL of each extract respectively. The plant showed highest total phenolic contents ranging from 29.5±0.49 to 112.5±0.36mg/g Gallic acid of extract, while the total flavonoid contents were estimated from3.08±0.02-85.4± 0.12mg/g Quercetin. The antioxidant activities of the all extracts, measured in terms of IC_{50} values were in the range of 53.05±1.98 to 211.5±31.06µg/mL. Nitric oxide scavenging ability exhibited their IC_{50} values from 26.3±5.51 to 121.3±5.32µg/mL. Ethanolic crude extract showed excellent result among all these fractions. GCMS analysis of ethanolic extract of *Carum carvi L* indicated the presence of several phytochemicals such as monoterpenes, unsaturated fatty acids, furan derivatives, phenolic and flavonoid contents.

Keywords: *Carum carvi L.* (*CC*), ethanolic extract(EE), ethyl acetate fraction (EAF), hexane fraction (HF), aqueous fraction (AQF), alpha-amylase (AALS), alpha-glucosidase (AGLS), half inhibitory concentration (IC_{50}), diabetes (DM)

INTRODUCTION

The prevalence rate of diabetes and obesity is expanding globally at an alarming rate due to alterations in present day standard of living (Tarling *et al*., 2008; Zimmet *et al*., 2001). According to the International Diabetes Federation (IDF) in 2017, approximately 451 million people were suffering from diabetes worldwide and this number is expected to exceed 693 million by the year 2045 (Cho *et al*., 2018). Diabetes mellitus (DM), one exists as insulin-dependent diabetes (type 1) and the other is non-insulin-dependent diabetes type 2 (Bharti *et al*., 2018). Type 1 is treated by injections of insulin regularly. 90% of diabetic people develop diabetes type 2 and management for this kind is not very easy, primary choice of remedy is through diet, controlling weight and management by physical activity (ADA, 2016; Chamberlain *et al*., 2016; Apostolidis and Lee, 2010). However, uncertainty of elevated level of blood glucose remain persists and managed by sulfonylureas, biguanides, thiazolidinediones, meglitinides, dipeptidyl peptidase IV inhibitors, has got side effects on human health (Chaudhury *et al*., 2017).

Literature revealed another management of DM is inhibiting digestive enzymes α-amylase and α-glucosidase (Kim *et al*., 2018). Free radical abundance may also suppress the insulin release in diabetes. Natural drugs have strong free radical scavenging ability to control oxidative. Some current reports indicated that antioxidants of the plasma have been exhausted in diabetic patients (Gutteridge, 1995). The oxidative stress and subsequent damage of tissue provide assurance in the pathogenesis of disease and therefore antioxidant treatment is used to neutralize the free radicals (Said *et al*., 2008). The natural activities of plant drugs are associated with the biochemical composition especially with those plants which are rich in phenolic and flavonoid contents, as they frequently indicate positive results in combating diabetes (Modak *et al*., 2007).

Carum carvi L. which is commonly known as Caraway belongs to the family *Apiaceae* (Agrahari and Singh, 2014). This plant is traditionally being used in different systems of medicine in Pakistan, India and China. CC is used as a carminative and for the treatment of spasmodic gastrointestinal problems, indigestion lack of appetite and dyspepsia (Thompson Coon and Ernst, 2002; Johri, 2011). The plant has shown antidiabetic, anti-oxidant, anti-carcinogenic and anti-bacterial activities. It is also being utilized as diuretic and expectorant and for increasing maternal milk and dysmenorrhea (Lahlou *et al*., 2007).

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The different chemicals has been isolated from its *viz* are mainly monoterpenes along with saturated, unsaturated fatty acids, oxygenated sesquiterpenes, ketones, aldehydes and esters (Zheng *et al*., 1992). Flavonoids, linalool, anethole, and other polyphenolic compounds also reported from CC (Najda *et al*., 2008). To the Scientifics' data, there are no available studies that reveal the enzymatic based antidiabetic potential by using this source for crude and fractions of CC. Therefore the aim of study is to explore natural plant based antidiabetic drug inhibitors having fewer side effects, low price and are relatively less harmless in the management of diabetes.

MATERIALS AND METHODS

Chemicals

3,5 Dinitrosalicylicacid, p-Nitro phenyl-alpha-D-glucopyranoside, 1,1-diphenyl-2-picryl-hydrazil (DPPH), Butylated hydroxy toluene (BHT), Folin-Ciocalteu's phenol reagent, Quercetin, Ascorbic acid, were purchased from sigma Aldrich. Tetra hydrate potassium sodium tartrate, Sodium hydroxide, Magnesium chloride, Sodium carbonate, Starch solution, DMSO (Dimethyl sulfoxide), Acarbose (Glucobay), Methanol, Ethanol, Hexane, Ethyl acetate, Sodium nitroprusside, Phosphate-buffered saline, Potassium phosphate buffer, Sulphanilamide, N-(1-naphthyl) ethylene diamine di hydrochloride, Phosphoric acid, Sodium nitrite, Aluminum chloride, Quercetin, Hydrochloric acid. All chemicals used were of analytical grade.

Collection, identification and authentication of plant

Seeds of *Carum carvi L.* was purchased (500g) during the month of October 2015 from herbal market of Karachi District (center) and identified by Prof. Dr. Ghazala H. Rizwani, (Meritorious) Department of Pharmacognosy, Faculty of Pharmacy, University of Karachi, Karachi, Pakistan. Seeds were clean by eliminating the dirt particles and kept in shade dried for few days.

Extraction and fractionation

The 500g seeds were weighed and soaked with 1L ethanol in glass jar. The seeds in the bottle were taped up and set aside for a period of about 15 days with occasional trembling then filter off through Whatmann filter paper No.1. The filtrate was then evaporated on a rotary evaporator under reduced pressure and control temperature i.e. 50^{o}C to obtain crude extract of CC. The same procedure was repeated three times with the left over seeds and then combined all filtrate. After that 10g of crude ethanol extract were fractionate with different organic solvents from lower to high polarity (n-hexane, ethyl acetate and aqueous). The presence of all major phytochemicals n the plant extract and their fractions were detected as per the standard procedures (Yadav *et al* ., 2014).

Estimation of phenolic content

Spectrophotometry was used to determine total phenolic constituents by the application of a reported method using a reagent called Folin-Ciocalteu's phenol reagent (Wickramaratne *et al*., 2015; Stankovic, 2011). 0.1mL extract of 10 mg/mL composition was mixed with 0.1mL of the reagent and kept for 5min. 1.0mL of 7percent solution of Na_2CO_3 was also included into the mixture and then diluted by deionized water of 1.30mL volume. The mixture was kept in the dark for of 90 minutes, after that; at 750 nm the absorbance was taken. Gallic acid was used for quantitative estimation of total phenolic content which is expressed as Gallic acid in mg equivalents in 1gm extract.

Estimation of flavonoid content

Total flavonoid content was estimated using a slightly altered colorimetric method (Zhishen *et al*., 1999). An aliquot of 0.5mL volume of a sample solution diluted and prepared with the addition of distilled water and 5% $NaNO_2$ solution to 2mL and 0.15mL volume, respectively. Added 10% $AlCl_3$ solution to the one which is 0.15mL and kept it aside for 6 min. Likewise added solution of NaOH (4%) in 2mL volume solution. Make up the volume to 5mL with water, mixed and kept the mixture for 15 min. At 520 nm Absorbance was measured against blank i.e. only water sample. Standard compound was used for the quantitative estimation of total flavonoid content. The values were expressed as mg (milligrams) of Quercetin equivalent in 1grams. Mean values are taken and standard deviation for three repetitions are presented.

Assay for alpha amylase inhibition

Alpha amylase which was extracted from bovine pancreas and the inhibitory activity was performed according to the method described by Ademiluyi and Oboh (2013) with slight modifications. DNS reagent has been performed as per (Highley TL (1997). 0.5mL of 0.1M potassium phosphate buffer (pH 7.0) was added to the reaction mixture, containing 0.1mL of alpha amylase enzyme (10 mg/mL) and plant extract in concentration range 100-250µg/mL that was pre-incubated for 10 minutes at 37°C.This was followed by addition of 1.0mL of 1.0% soluble starch solution (prepared in 0.1M potassium phosphate buffer pH 7.0) and incubated at 37°C for 10 minutes. The reaction was completed with the addition of 1.0mL DNS reagent and placed for 5 minutes in boiling water bath, cooled to room temperature and diluted with 0.9mL distilled water. The absorbance was measured at 540 nm. Control samples were also prepared without any plant extract and compared with the test samples containing the plant extracts prepared with different solvents. For standard, Acarbose was used. The alpha-amylase inhibitory activity was calculated as % inhibition.

Percentage inhibition (%) = [Ac – As/Ac] × 100

Where

Ac = Absorbance of Control

As = Absorbance of Sample

Acarbose extraction

Stock standard solution of Acarbose (Glucobay) was prepared in a concentration of 5.0mg/mL with DMSO and diluted to 250µg/mL using 0.1M potassium phosphate buffer (pH 7.0).

Assay protocol for Alpha-glucosidase inhibition

For the determination of inhibitory activity of α-glucosidase, a standard method was used with some alterations (Apostolidis *et al*., 2006). Alpha-glucosidase which was extracted in 0.1 units per mL from green gram was solubilized in buffer solution of potassium phosphate (potassium phosphate 0.1 mol/ L, 3.2mmol/ Liter -$MgCl_2$ pH 7.0). To the same buffer solution p-nitrophenyl-alpha-D-glucopyranoside at 5mmol/L was dissolved and used as substrate. The reaction mixture contained 0.5mL buffer (pH 7.0, 0.1mol per Liter of potassium phosphate, -$MgCl_2$ 3.2 mmol per L), in concentrations of 100,150,200 and 250 µg per mL solution sample in 0.1mL enzyme and Dimethyl sulfoxide. Incubation time and temperature for keeping the mixture in water bath was 10 minutes at 37°Cfollowed by the addition of substrate solution 0.5mL in the reaction mixture for 20 min at a temperature 37°C. For the completion the reaction 1.0 mL of 20.0% Na_2CO_3 (Sodium carbonate) is added to it. Enzyme inhibitory activity was monitored by measuring the absorbance at 420 nm. Control samples were prepared devoid of plant extract. Acarbose was used as a standard. The inhibitory action for alpha-glucosidase was calculated as percent inhibition.

Percent inhibition (%) = [(Ac-As) /Ac] × 100
Where,
Ac = Absorbance of Control
As = Absorbance of Sample

Assay of DPPH (Radical-Scavenging-Activity)

The free radical scavenging activity was determined with 1,1-diphenyl-2-picryl-hydrazil (DPPH) by a slight manipulation of the method demonstrated by Gulcin *et al*., (2005). DPPH solution in methanol of 0.3mM was prepared and then in ten microliters of each sample of different concentrations (25 µg - 100 µg) was mixed with 1.0mL of DPPH solution. The reaction mixture was incubated at 37°C for 30 min. Absorbance was measured at a wavelength of 515 nm by spectrophotometer and the percent radical scavenging activity was calculated in parallel with a control solution. Ascorbic acid was used as a Standard.

DPPH inhibitory activity (%) = ((Absorbance of Control - Absorbance of Sample)/Absorbance of Control) × 100

Assay of Nitric oxide (Scavenging activity)

Griess reagent was used to measure nitrite ions, which were produced in the reaction of sodium nitroprusside and oxygen which led to the formation of Nitric oxide. The assay procedure was used with some modifications described by Green *et al*. (1982). In this test, 1.5ml of sodium nitroprusside (10mM) was added in phosphate-buffered saline (0.1 M, pH 7.0), mixed with the extract (50-200 µg per mL) and incubated at 30°C for 120 min. The reaction mixture, with an equivalent volume of buffer, without the extracts served as control. Following the incubation period, 2.5 mL of Griess reagent (1% sulphanilamide, 0.1% N-(1-naphthyl) ethylene diamine hydrochloride) in 2% H_3PO_4 was added. The absorbance was read spectrophotometrically at 546 nm. Butylated Hydroxy Toluene (BHT) was used as standard. The free radical inhibitory activity was determined by %inhibition.

Gas chromatography- Mass spectrometry (GC-MS)

The analysis was performed by an Agilent equipment 5975series GC attached through (an Agilent) 7890. Helium was used as a carrier gas at a flow rate of 1.0 mL / min .Temperature of injector was fixed at 280°C. The early oven temperature at 60°C which was automated rose to 180°C at the rate of 5°C/min for15 minutes and then raised to 300°C at the rate of 7°C / min for 15 minutes with a hold up time of 1: 3653 min. The mass spectrometer was worked at (70ev) electron ionization mode and electron multiplier voltage at 1588 v. The complexes were recognized by direct association of the fragmentation pattern, retention times and mass spectral data through (national institute of standards and Technology) (NIST) Library (Jadhav *et al*., 2014).

STATISTICAL ANALYSIS

Results were recorded as mean ± S.D values of triplicates. IC_{50} values were calculated by various concentrations in comparison to their respective positive control by absorbance measurement. IC_{50} values were measured from curves obtained by linear regression.

RESULTS

The weight of crude extract and its fractions was given in table 1.

Table 1: Weights of fraction of *CC* seeds

Name of fractions	Weight of fractions(g)
Ethyl acetate	3.5g
Hexane	1.22g
Aqueous	2.8g

Estimation of phenolic and flavonoid content (TPC and TFC)

The amount determined for phenolic and flavonoid contents is given in table 2 and the amount were determined as triplicate .The highest amount of TPC and TFC of plant (*CC*) in its extract and different fractions were observed to be 112.5±0.36, 91.6±0.29,57.5±0.21and 29.5 ±0.49mg/g Gallic acid for aqueous, ethyl acetate, ethanol and hexane respectively. While the highest flavonoid contents were observed 85.4±0.124, 81.1± 0.16,

49.6 ± 0.93, and 3.08mg/g Querecitin for aqueous, ethanol, ethyl acetate and hexane respectively.

Table 2: Quantitative Estimation of Total Phenolic Content (TPC) (mg/g Gallic Acid) and Total Flavonoids Content (TFC) (mg/g Quercetin) of seeds (*CC*)

Samples	TPC (mg/g Gallic Acid)	TFC contents (mg/g Quercetin)
Ethanol	57.5 ± 0.21	81.1± 0.16
Ethyl acetate	91.6 ± 0.29	49.6 ± 0.93
Hexane	29.5 ±0.49	3.08 ± 0.02
Aqueous	112.5 ±0.36	85.4 ± 0.12

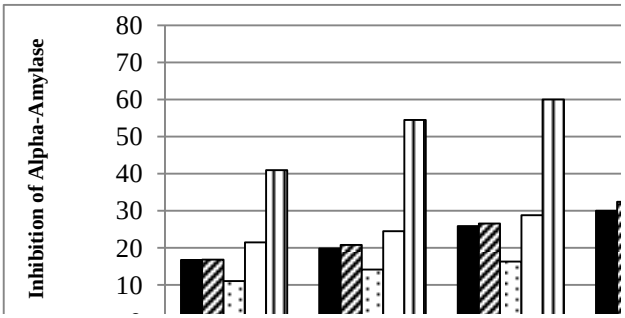

Fig. 1: Results are given as mean of α-amylase inhibitory activity of extract and fractions of (*CC*) at different concentration EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Acarbose=Standard

Alpha amylase and alpha glucosidase inhibitory activity

The ethanolic crude extract showed less inhibition on AALS which is found to be 30.0% at conc. 250µg/mL (fig. 1) with its IC_{50} value of 467.3±15.3µg/mL (fig. 2) which is extensively higher than standard i.e. Acarbose, even though the same conc. showed significant inhibition on AGLS i.e.64.7% (fig. 3) with a minimum IC_{50} value i.e. 72±8.81µg/mL (fig. 4) which is far less than Acarbose. EAF and AQF show substantial inhibition 32.4 % and 31.5% on α -amylase at conc.250µg/mL (fig. 1) and their IC_{50} values were calculated as 421.4±7.8 and 516.4±165.6µg/mL, respectively (fig. 2). Furthermore highest inhibition of EAF and AQF was observed on AGLS (83.4% and 82.3%) at conc. 250µg/mL (fig. 3). The IC_{50} values were determined as147±2.49 and 129±10.3µg /mL respectively (fig. 4). AQF of ethanolic extract showed least IC_{50} values which are less than Acarbose (fig. 4). HF indicated very less inhibition on α -amylase (19.4 %) at conc. 250µg/mL (fig. 1) with its IC_{50}value 810±5.71 (fig. 2) while, extensive inhibition was observed on α-glucosidase (39.1%) at the same conc. with its highest IC_{50}value calculated as 307.0±11.42µg/mL (fig. 4).

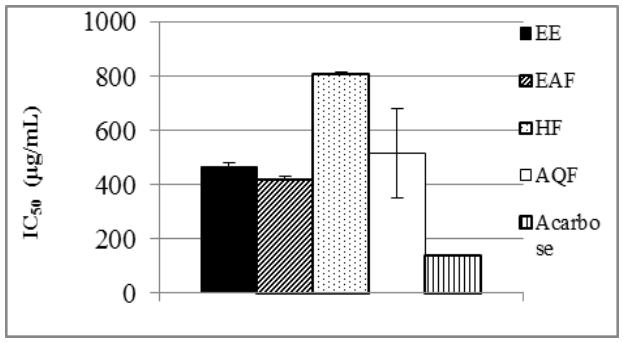

Fig. 2: Results are given as mean ± S.D of IC_{50} (µg/mL) values of α- amylase inhibitory activity of extract and fractions of (*CC*) EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Acarbose=Standard

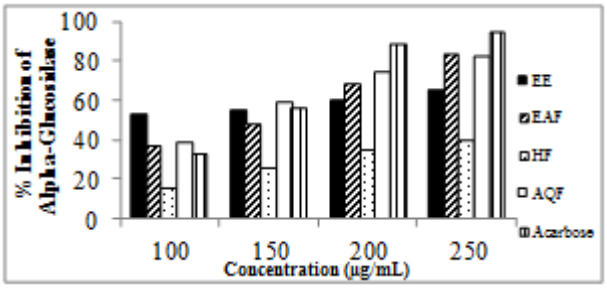

Fig. 3: Results are given as mean of α-glucosidase inhibitory activity of extract and fractions of (*CC*) at different concentration EE=Ethanolic extract, EAF=Ethyl acetate

Table 3: Phytoconstituents identified from ethanolic extract of (*CC*) by GC-MS analysis

Peak no	RT(min)	Name of compound	Molecular formula	Molecular weight	Peak area%
02	12.817	2-Furanmethanol,5-ethenyl tetrahydroα,α,5-trimethyl-,cis-	$C_{10}H_{18}O_2$	170	0.24
03	13.210	Benzaldehyde,4-(1-methylethyl)	$C_{10}H_{12}O$	148	1.32
07	14.705	Thymol	$C_{10}H_{14}O$	150	0.81
11	15.55	2(3H)-Benzofuranone hexahydro-3-methylene	$C_9H_{12}O_2$	152	0.20
16	20.91	Phenol, 2-methoxy .5 – l – propenyl	$C_{10}H_{12}O_2$	164	0.36
20	30.79	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	0.89
21	32.38	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.34
22	42.13	9, 12-octadecadienoic acid (z,z)	$C_{18}H_{32}O_2$	280	1.41
23	42.44	Cis. Vaccenic acid	$C_{18}H_{34}O_2$	282	1.19
24	44.53	5-Benzofuranacetic acid, 6-elthenyl-2, 4, 5, 6, 7 , 7a-hexahydro-7-a-hydroxyl-3, 6-dimethyl-d-methylene-2-oxo-methyl ester	$C_{16}H_{20}O_5$	292	1.19

fraction, HF=Hexane fraction, AQF= Aqueous fraction, Acarbose=Standard

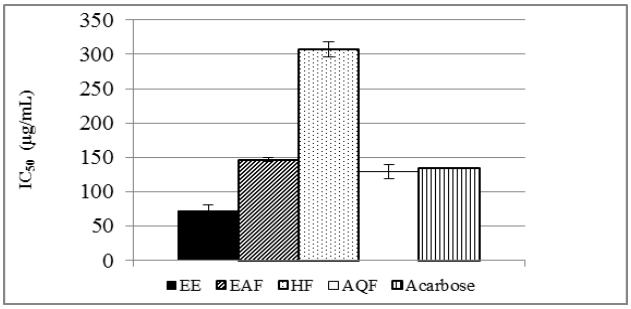

Fig. 4: Results are given as mean ± S.D of IC_{50} (µg/mL) values of α-glucosidase inhibitory activity of extract and fractions of (*CC*)

EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Acarbose=Standard

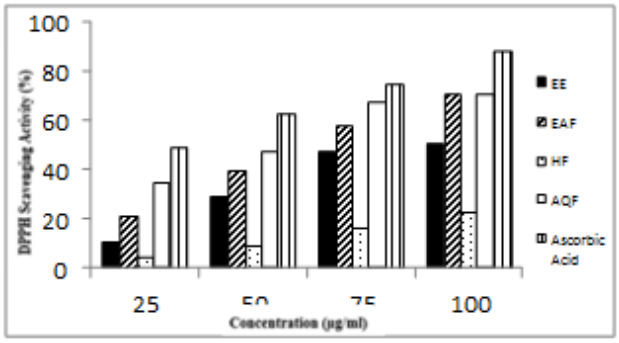

Fig. 5: Results are given as mean of DPPH scavenging activity of extract and fractions of (*CC*) at different concentration

EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Ascorbic acid=Standard

Antioxidant by DPPH

EAF and AQF showed significant antioxidant activity and the inhibition was 21.0–70.0 and 34.2-70.5% (fig. 5) and IC_{50} values as 67.2±1.91 and 53.05±1.98µg/mL at conc.25-100µg/mL respectively (fig. 6). On the other hand moderate scavenging activity was observed in EE and its inhibition was in the range from 10.5-50.8%, at conc.25-100µg/mL (fig. 5) along with IC_{50} of 90.4±3.52µg/mL (fig. 6). Although least inhibition in HF was observed which varies from 4.3-22.4-% at conc. 25-100µg/mL with its IC_{50} 211.5± 31.06/mL

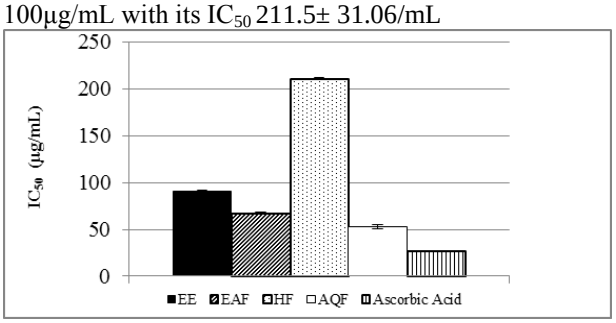

Fig. 6: Results are given as mean ± S.D of IC_{50} (µg/mL) values of (DPPH) scavenging activity of extract and fractions of (*CC*)

EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Ascorbic acid=Standard

Antioxidant by Nitric oxide

Scavenging activity of reacting nitrogen species (RNS) of Plant (*CC*) and its fractions (EE, EAF, HF and AQF) are given in (fig. 7) which suggest that HF, AQF and EAF show stronger quenching abilities of RNS and inhibition of nitrogen in the range from 38.3-68.2%, 47.0-66.2% and 31.8-63.4% at conc. 25-200µg/mL and IC_{50} values of 88.35±2.29, 26.3±5.51and 101.0±1.44µg/mL (fig. 8) respectively. While in EE moderate nitrogen Scavenging activity was observed which ranges from 33.0-58.5% at conc. 25-200µg/mL (fig. 7) with IC_{50} value 121.3±5.32µg/mL (fig. 8) which is higher than the standard, butylated hydroxyl toluene. Whereas AQF showed IC_{50} 26.3µg/mL which is less than BHT (fig. 8).

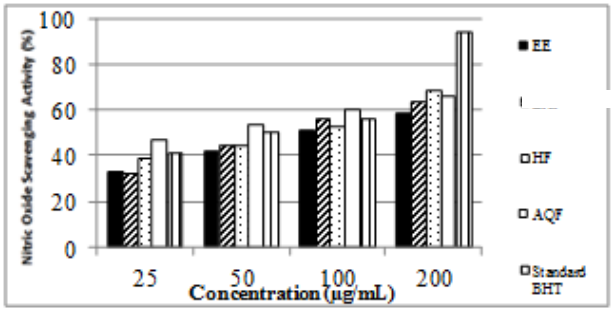

Fig. 7: Results are given as mean of Nitric oxide scavenging activity of extract and fractions of (*CC*) at different concentration.

EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Butylated hydroxyl toluene (BHT) =Standard

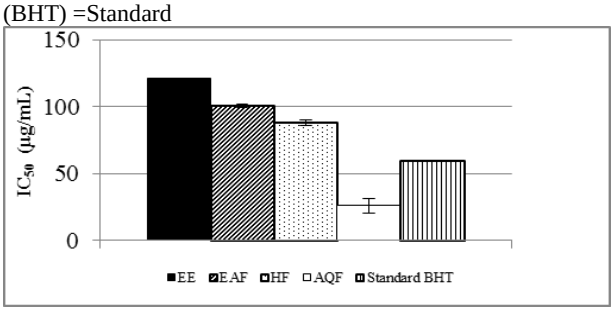

Fig. 8: Results are given as mean ± S.D of IC_{50} (µg/mL) values (Nitric Oxide) scavenging activities of extract and fractions of (*CC*).

E=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AQF= Aqueous fraction, Butylated hydroxyl toluene (BHT) =Standard

GC-MS analysis

Table 3 depicts the compounds of the ethanolic extract of (*CC*) by GC-MS analysis having the most prominent peaks which are identified as (I) 2-furan methanol, 5-ethenyl tetra hydro –α, α 5-trimethyl-cis (II) benzaldehyde 4-1- methyl ethyl- (III) thymol (IV) 2(3H)-Benzofuranone, hexahydro-3-methylene (V) phenol, 2 methoxy-5-1-propenyl (E) (VI) hexadecenoic acid methyl ester, (VII) n-hexadecenoic acid (VIII) 9, 12-octadecanoic acid(IX) cis vaccenic acid (X) 5-Benzofuran acetic acid, 6ethynl- 2,4,5,6,7,7a-hexahydro-7a-hydroxy-3,6-dimethyl-α–methylene -2- oxo methyl ester. The mass spectra of these compounds as shown in (fig. 9a).

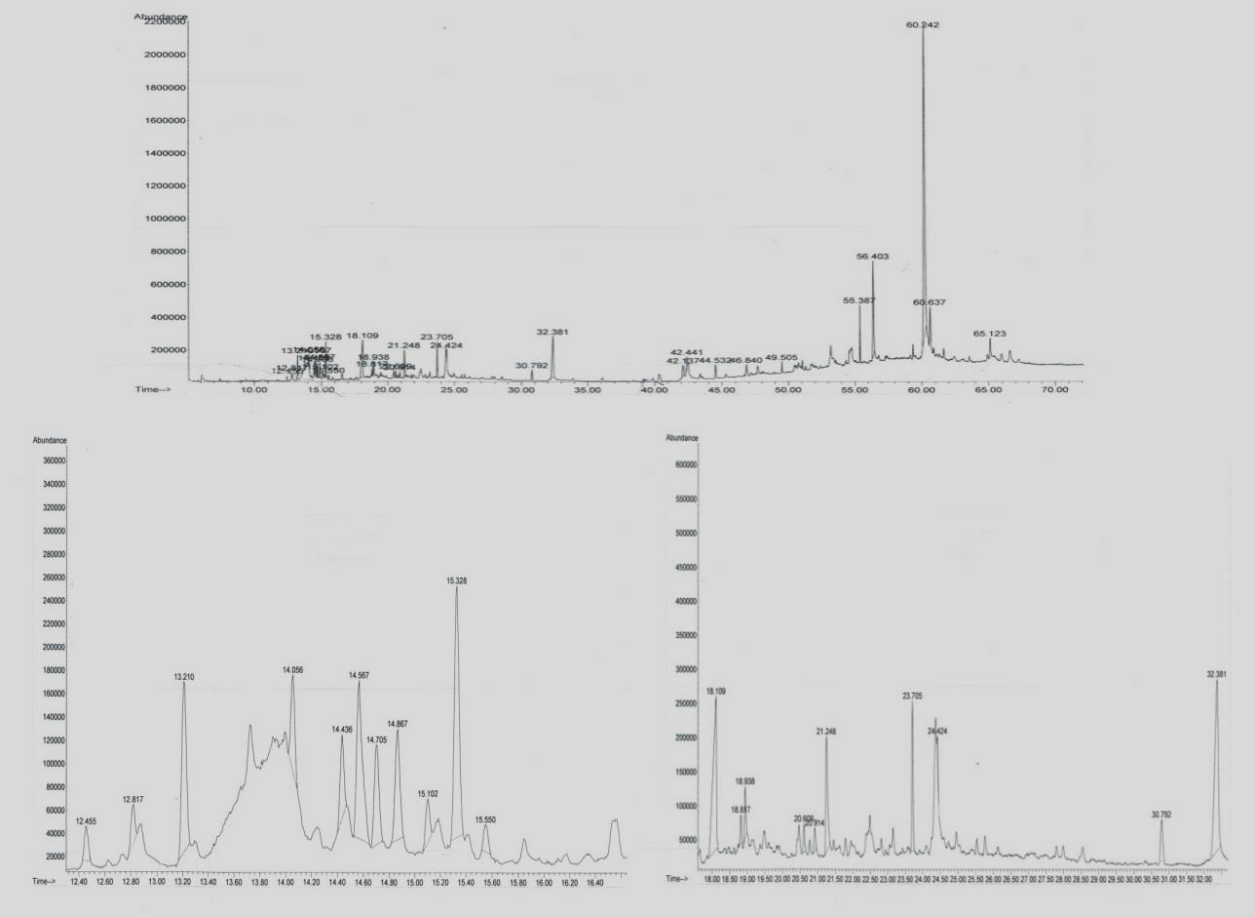

Fig. 9: GC-MS chromatogram of ethanolic extract of (*CC*)

DISCUSSION

In the present study, enzyme inhibition activity was detected in the plant seed extract and its various fractions. Free radical scavenging activity by reactive oxygen species and reactive nitrogen species were performed along with estimation of phenol and flavonoids components.

In this research, we found that crude seeds extract and its fractions scavenging free radicals ability to scavenge reactive oxygen with IC_{50} values were considerable as compared to standard Ascorbic acid. While AQF showed strongest ability to scavenge reactive nitrogen with IC_{50} value is significant as compared to standard butylated hydroxyl toluene (fig. 8). This activity is attributed due to higher amount of phenolic and flavonoid contents which are found to be 112.5±0.368 mg/g of Gallic acid of dry extract similarly estimation of flavonoid content observed as 85.4±0.124mg/g Querecitin of dry extracts. This research study also indicated that there is a strong relationship between the phenolic and antioxidant activity which is in conformity with the several studies that are reported in the literature (Patel *et al*., 2011). Phenolic and flavonoid compounds diminish the oxidative stress by providing an equilibrium between oxidants and antioxidants due to their decreasing metal chelating and free radical scavenging properties by donating hydrogen from hydroxyl groups (Gonçalves and Romano, 2017).

Whereas, antidiabetic activity by inhibiting digestive enzymes such as AALS and AGLS the plant exhibited noticeable inhibition on α-amylase while, EE and AQF showed strongest inhibition on α-glucosidase. Their IC_{50} values were 72±8.81 and 129±10.3µg/mL (fig. 4) respectively, which are less than the standard Acarbose. Good inhibition with lowest IC_{50} values was observed in EE due to the presence of higher flavonoid contents which are reported as potent antioxidant, anti-inflammatory properties, improve glucose and lipid metabolism (Testa *et al*., 2016). EAF also indicated its IC_{50} values are close to the Acarbose. Enzyme inhibitory action especially of digestive enzymes α-amylase and α-glucosidase are involved in the carbohydrate digestion and extensively diminish the postprandial hyperglycemia (Ali *et al*., 2006). These results proved that these digestive enzymes play an important role in the management of diabetes by delayed absorption of carbohydrate through digestive enzymes inhibitors (McCue *et al*., 2005).

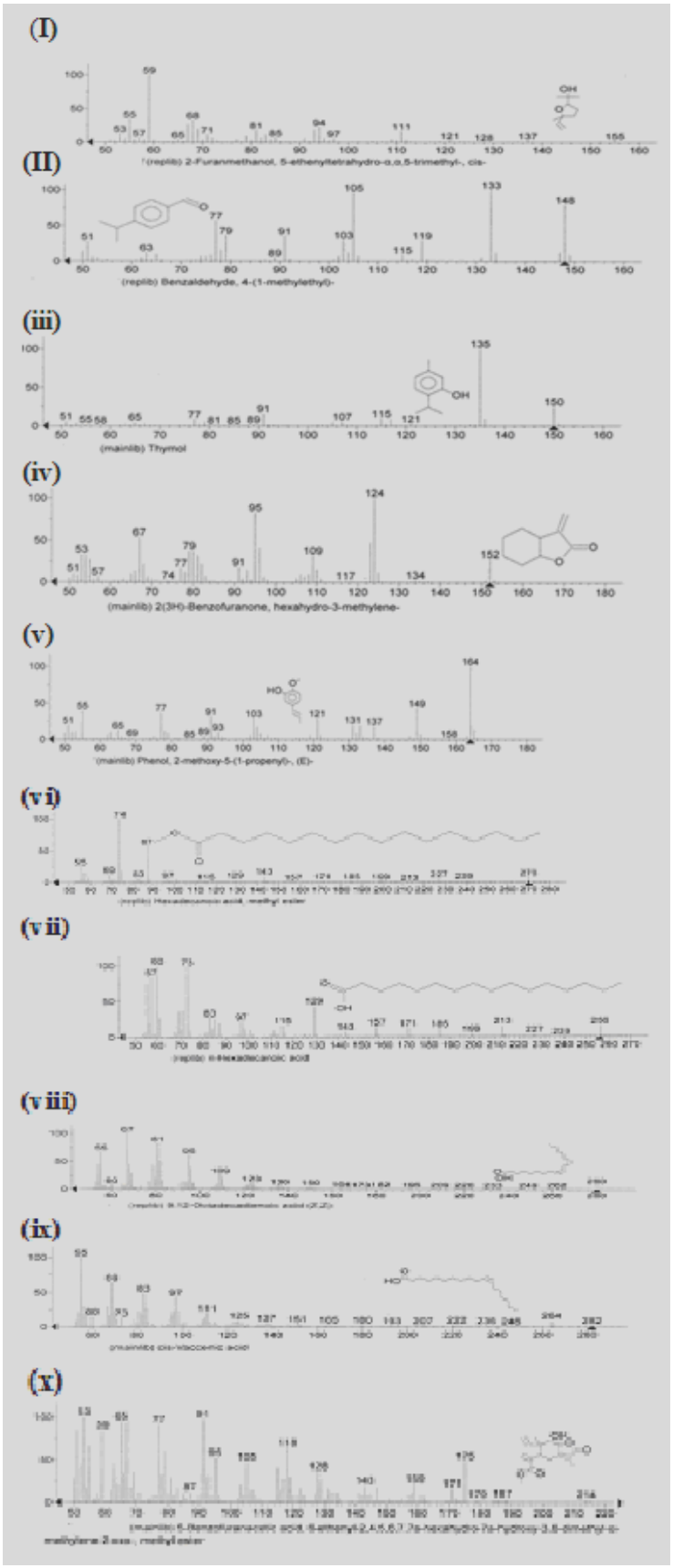

Fig. 9a: Mass spectrum of phytoconstituents identified by GC-MS in ethannolic extract of *Carum carvi* L**.**

The GCMS analysis of crude ethanol extract was also indicated the presence of some other valuable phenolic and flavonoids phytoconstituents as indicated in table (3). A monoterpene phenolic compound Thymol which seems likely to be responsible for anti-diabetic activity (Saravanan and Pari, 2015)

Moreover, bioactive fatty acid methyl esters (FAMEs) and other benzene derivatives have anti-diabetic related activities (Berraaouan *et al* 2013). Cis vaccenic acid was reported for hypolipidaemic and antihypertensive activities (Bhattacharya *et al*., 2014).While some furan derivatives showed anti-diabetic activity (Suresh Babu, 2012). GC-MS spectrum showed the presence of these above compounds in ethanolic crude extract.

CONCLUSION

The antidiabetic activity of seeds of *Carum carvi* L. via digestive enzyme inhibitory model was performed. The presence of phenolic and flavonoid contents was estimated and their antioxidant potential was correlated. Our results indicated that the seeds of CC are rich in phenolic contents, fatty acid and furan derivatives along with other phytoconstituents making it of a useful potential against antioxidant and anti-diabetic activities. As a result of extensive research exercise culinary herb CC proved to be a potent antidiabetic and antioxidant drug which is also correlated with its anti-obesity potential. As a consequence, the obtained results may be significant enough to new findings towards other members of condiments which are used as food.

REFERENCES

ADA (2016). Standards of medical care in diabetes: Summary of revisions. *Diabetes care*, **39**(Suppl. 1): S4-S5.

Ademiluyi AO and Oboh G (2013). Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) *in vitro*. *Exp. Toxicol. Pathol.*, **65**(3): 305-309.

Agrahari P and Singh DK (2014). A review on the pharmacological aspects of Carumcarvi. *J. Biol. Earth Sci.*, **4**(1): 1-13.

Ali H, Houghton P and Soumyanath A (2006). α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to Phyllanthusamarus. *J. Ethnopharmacol.*, **107**(3): 449-455.

Apostolidis E and Lee C (2010). *In vitro* potential of Ascophyllumnodosum phenolic antioxidant mediated α-glucosidase and α-amylase inhibition. *J. Food Sci.*, **75**(3): 97-102.

Apostolidis E, Kwon YI, Shetty K, Apostolidis E and Kwon YI (2006). Potential of cranberry-based herbal synergies for diabetes and hypertension management. *Asia Pac. J. Clin. Nutr.*, **15**(3): 433-441.

Babu SP and Suresh Babu K (2012). Synthesis and antidiabetic activity of 2, 4-disubstituted furan derivatives. *Der. Pharma. Chemica.*, **4**(6): 2178-2186.

Berraaouan A, Abid S and Bnouham M (2013). Antidiabetic oils. *Curr. Diabetes Rev.*, **9**(6): 499-505.

Bharti SK, Krishnan S, Kumar A and Kumar A (2018). Antidiabetic phytoconstituents and their mode of action on metabolic pathways. *Ther. Adv. Endocrinol. Metab.*, **9**(3): 81-100.

Bhattacharya A, Ghosh G, Agrawal D, Sahu PK, Kumar S and Mishra SS (2014). GC-MS profiling of ethanolic extract of *Moringa oleifera* leaf. *Int. J. Pharm. Bio. Sci*, **5**(4): 263-275.

Chamberlain JJ, Rhinehart AS, Shaefer CF and Neuman A (2016). Diagnosis and management of diabetes: Synopsis of the American Diabetes Association Standards of Medical Care in Diabetes. *Ann. Intern. Med.*, **164**(8): 542-552.

Chaudhury A, Duvoor C, Dendi R, Sena V, Kraleti S, Chada A and Kuriakose K (2017). Clinical review of antidiabetic drugs: Implications for type 2 diabetes mellitus management. *Front. Endocrinol*., **8**: 6.

Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A and Malanda B (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.*, **138**(2018): 271-281

Gonçalves S and Romano A (2017). Inhibitory properties of phenolic compounds against enzymes linked with human diseases. Chapter 6. *In*: Phenolic compounds-biological activity. Rijeka, IntechOpen Limited, Croatia, pp.99-118.

Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS and Tannenbaum SR (1982). Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Anal. Biochem.*, **126**(1): 131-138.

Gulcin I, Alici HA and Cesur M (2005). Determination of in vitro antioxidant and radical scavenging activities of propofol. *Chem. Pharm. Bull.*, **53**(3): 281-285.

Gutteridge, J. (1995). Lipid peroxidation and antioxidants as biomarkers of tissue damage. *Clin. Chem*, **41**(12): 1819-1828.

Highley TL (1997). Carbohydrolase assays. Chapter 12: Methods in Plant Biochemistry and Molecular Biology. CRC Press, Boca Raton, pp.309-321.

Jadhav V, Kalase V and Patil P (2014). GC-MS analysis of bioactive compounds in methanolic extract of *Holigarna grahamii* (wight) Kurz. *Int. J. Herb. Med*, **35**(24): 35-39.

Johri R (2011). Cuminumcyminum and Carumcarvi: An update. *Pharmacog. Rev*, **5**(9): 63-72.

Kim HH, Kang YR, Lee JY, Chang HB, Lee KW, Apostolidis E and Kwon YI (2018). The postprandial anti-hyperglycemic effect of pyridoxine and its

derivatives using *in vitro* and *in vivo* animal models. *Nutrients*, **10**(3): 285.

Lahlou S, Tahraoui A, Israili Z and Lyoussi B (2007). Diuretic activity of the aqueous extracts of *Carum carvi* and *Tanacetum vulgare* in normal rats. *J. Ethnopharmacol.*, **110**(3): 458-463.

McCue P, Kwon YI and Shetty K (2005). Anti-diabetic and anti-hypertensive potential of sprouted and solid-state bioprocessed soybean. *Asia Pac. J. Clin. Nutr*, **14**(2): 145-152.

Modak M, Dixit P, Londhe J, Ghaskadbi S and Devasagayam TPA (2007). Recent advances in Indian herbal drug research guest editor: Thomas Paul Asir Devasagayam Indian herbs and herbal drugs used for the treatment of diabetes. *J. Clin. Biochem. Nutr*, **40**(3): 163-173.

Najda A, Dyduch J and Brzozowski N (2008). Flavonoid content and antioxidant activity of caraway roots (*Carum carvi* L.). *Veg. Crops Res. Bull.*, **68**: 127-133.

Patel D, Kumar R, Prasad S, Sairam K and Hemalatha S (2011). Antidiabetic and *in vitro* antioxidant potential of *Hybanthus enneaspermus* (Linn) F. Muell in streptozotocin induced diabetic rats. *Asian Pac. J. Tropical Biomed.*, **1**(4): 316-322

Said O, Fulder S, Khalil K, Azaizeh H, Kassis E and Saad B (2008). Maintaining a physiological blood glucose level with 'glucolevel', a combination of four anti-diabetes plants used in the traditional Arab herbal medicine. *Evid. Based Complement. Alternat. Med*, **5**(4): 421-428.

Saravanan S and Pari L (2015). Role of thymol on hyperglycemia and hyperlipidemia in high fat diet-induced type 2 diabetic C57BL/6J mice. *Eur. J. Pharmacol.*, **761**: 279-287.

Stankovic MS (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. extracts. *Kragujevac J. Sci.*, **33**(2011): 63-72.

Tarling CA, Woods K, Zhang R, Brastianos HC, Brayer GD, Andersen RJ and Withers SG (2008). The search for novel human pancreatic α-amylase inhibitors: High-throughput screening of terrestrial and marine natural product extracts. *Chem. Bio. Chem.*, **9**(3): 433-438.

Testa R, Bonfigli A, Genovese S, De Nigris V and Ceriello A (2016). The possible role of flavonoids in the prevention of diabetic complications. *Nutrients*, **8**(5): 310-323.

Thompson Coon J and Ernst E (2002). Herbal medicinal products for non-ulcer dyspepsia. *Aliment. Pharmacol. Ther.*, **16**(10): 1689-1699.

Wickramaratne MN, Gunatilake L, Anuradha ND, Godavillathanna A, Perera M and Nicholas I (2015). Antioxidant activity and antibacterial activity of *Walidda antidysenterica*. *J. Pharmacog. Phytochem.*, **4**(2): 121-126.

Yadav M, Chatterji S, Gupta SK and Watal G (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *Int. J. Pharm. Pharm. Sci.*, **6**(5): 539-542.

Zheng GQ, Kenney PM and Lam LK (1992). Anethofuran, carvone, and limonene: Potential cancer chemoprotective agents from dill weed oil and caraway oil. *Planta Medica.*, **58**(4): 338-341.

Zhishen J, Mengcheng T and Jianming W (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.*, **64**(4): 555-559.

Zimmet P, Alberti K and Shaw J (2001). Global and societal implications of the diabetes epidemic. *Nature*, **414**(6865): 782-787.