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 IC₅₀ 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 IC₅₀ values were in the range of 53.05±1.98 to 211.5±31.06µg/mL. Nitric oxide scavenging ability exhibited their IC₅₀ 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 (IC₅₀), 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 Apioaceae (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). 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 Scientists’ 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 Dinitosalicylic acid, 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 grade. Temperature i.e. 50°C. 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°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 in 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 Na2CO3 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% NaNO2 solution to 2mL and 0.15mL volume, respectively. Added 10% AlCl3 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). 0.1mL 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 -MgCl2 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, -MgCl2 3.2 mmol per L), in concentrations of 100,150,200 and 250 µg per mL solution sample in 0.1mL enzyme and 37°C followed 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% Na2CO3 (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% H3PO4 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. IC50 values were calculated by various concentrations in comparison to their respective positive control by absorbance measurement. IC50 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

<table>
<thead>
<tr>
<th>Name of fractions</th>
<th>Weight of fractions(g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>3.5g</td>
</tr>
<tr>
<td>Hexane</td>
<td>1.22g</td>
</tr>
<tr>
<td>Aqueous</td>
<td>2.8g</td>
</tr>
</tbody>
</table>

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±0.29,29.5±0.21 and 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,
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49.6 ± 0.93, and 3.08µg/mL Quercetin 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)

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

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 IC₅₀ 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 IC₅₀ value i.e. 72±8.1µg/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 IC₅₀ 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 IC₅₀ values were determined as147±2.49 and 129±10.3µg /mL respectively (fig. 4). AOF of ethanolic extract showed least IC₅₀ 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 IC₅₀ value 810±5.71 (fig. 2) while, extensive inhibition was observed on α-glucosidase (39.1%) at the same conc. with its highest IC₅₀ value calculated as 307.0±11.42µg/mL (fig. 4).

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

<table>
<thead>
<tr>
<th>Peak no</th>
<th>RT(min)</th>
<th>Name of compound</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Peak area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>02</td>
<td>12.817</td>
<td>2-Furanmethanol,5-ethyl tetrahydroo,a,5-trimethyl-,cis-</td>
<td>C₁₀H₁₄O₂</td>
<td>170</td>
<td>0.24</td>
</tr>
<tr>
<td>03</td>
<td>13.210</td>
<td>Benzaldehyde,4-(1-methylethyl)</td>
<td>C₁₀H₁₀O</td>
<td>148</td>
<td>1.32</td>
</tr>
<tr>
<td>07</td>
<td>14.705</td>
<td>Thymol</td>
<td>C₁₀H₁₄O</td>
<td>150</td>
<td>0.81</td>
</tr>
<tr>
<td>11</td>
<td>15.55</td>
<td>2(3H)-Benzofuranone hexahydro-3-methylene</td>
<td>C₆H₁₄O₂</td>
<td>152</td>
<td>0.20</td>
</tr>
<tr>
<td>16</td>
<td>20.91</td>
<td>Phenol, 2-methoxy .5 – 1 – propenyl</td>
<td>C₆H₁₂O₂</td>
<td>164</td>
<td>0.36</td>
</tr>
<tr>
<td>20</td>
<td>30.79</td>
<td>Hexadecanoic acid, methyl ester</td>
<td>C₁₇H₃₄O₂</td>
<td>270</td>
<td>0.89</td>
</tr>
<tr>
<td>21</td>
<td>32.38</td>
<td>n-Hexadecanoic acid</td>
<td>C₁₆H₃₃O₂</td>
<td>256</td>
<td>6.34</td>
</tr>
<tr>
<td>22</td>
<td>42.13</td>
<td>9, 12-octadecadienoic acid (z,z)</td>
<td>C₁₈H₃₄O₂</td>
<td>280</td>
<td>1.41</td>
</tr>
<tr>
<td>23</td>
<td>42.44</td>
<td>Cis. Vaccenic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>282</td>
<td>1.19</td>
</tr>
<tr>
<td>24</td>
<td>44.53</td>
<td>5-Benzofuranacetic acid, 6-ethenyl-2, 4, 5, 6, 7, 7a- hexahydro-7-a-hydroxyl-3, 6-dimethyl-d-methylene-2-oxo-methyl ester</td>
<td>C₁₆H₂₃O₅</td>
<td>292</td>
<td>1.19</td>
</tr>
</tbody>
</table>
fraction, HF=Hexane fraction, AWF= Aqueous fraction, Acarbose=Standard

Fig. 4: Results are given as mean ± S.D of IC₅₀ (µg/mL) values of α-glucosidase inhibitory activity of extract and fractions of (CC)
EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AWF= 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, AWF= Aqueous fraction, Ascorbic acid=Standard

Antioxidant by DPPH
EAF and AWF showed significant antioxidant activity and the inhibition was 21.0–70.0 and 34.2–70.5% (fig. 5) and IC₅₀ 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 IC₅₀ 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 IC₅₀ 211.5± 31.06mL

Fig. 6: Results are given as mean ± S.D of IC₅₀ (µg/mL) values of (DPPH) scavenging activity of extract and fractions of (CC)
EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AWF= 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 AWF) are given in (fig. 7) which suggest that HF, AWF 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 IC₅₀ 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 IC₅₀ value 121.3±5.32µg/mL (fig. 8) which is higher than the standard, butylated hydroxyl toluene. Whereas AWF showed IC₅₀ 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, AWF= Aqueous fraction, Butylated hydroxyl toluene (BHT)=Standard

Fig. 8: Results are given as mean ± S.D of IC₅₀ (µg/mL) values (Nitric Oxide) scavenging activities of extract and fractions of (CC).
EE=Ethanolic extract, EAF=Ethyl acetate fraction, HF=Hexane fraction, AWF= 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 tetrahydro –α, α 5-trimethyl-cis (II) benzaldehyde 4-1- methyl ethyl (III) thymol (IV) 2(3H)-Benzo furanone, hexahydro-3-methylene (V) phenol, 2 methoxy-5-1-propenyl (E) VI hexadecenoic acid methyl ester, (VII) n-hexadecenoic acid (VIII) 9, 12-octadecenoic acid(IX) cis vaccenic acid (X) 5-Benzofuran acetic acid, 6ethynyl - 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).
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 Quercetin 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 $\alpha$-amylase while, EE and AQF showed strongest inhibition on $\alpha$-glucosidase. Their IC$_{50}$ values were 72±8.81 and 129±10.3 µg/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 $\alpha$-amylase and $\alpha$-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. 9: GC-MS chromatogram of ethanolic extract of (CC)
Fig. 9a: Mass spectrum of phytoconstituents identified by GC-MS in ethanolic 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 monoterpenic 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 anetholic 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


