Phytoconstituents Investigation, Anti-diabetic and Anti-dyslipidemic Activities of *Cotoneaster horizontalis* Decne Cultivated in Egypt

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Abstract: Quantitative determination of the total polyphenols and flavonoids of aerial parts of *Cotoneaster horizontalis* Decne family Rosaceae was performed colorimetrically using Folin-Ciocalteu and aluminum tri-chloride methods respectively. The concentration of total polyphenols expressed as Gallic acid equivalent (GAE) was 14± 0.16 mg /g plant extract GAE, while the concentrations of flavonoids and flavonol contents expressed as rutin equivalent (RE) were 6.8± 0.76 and 2.2± 00 mg /g plant extract RE respectively. HPLC analysis of the total polyphenolic contents of aerial parts of the plant revealed the presence of eight compounds and pyrogallol was the highest concentration compound (1029.57 mg/100g dried plant powder), while HPLC analysis of total flavonoids showed the presence of three flavonoids and luteolin was the major compound (9.20mg/100g dried plant powder).GLC analysis of un-saponifiable matters (USM) of the aerial parts of the plant indicated the presence of n-triacontane (17.11%) , β-sitosterol (8.48%) and α-amyrine (14.35%) as the major hydrocarbon , sterol and triterpene respectively. GLC analysis of fatty acid methyl esters revealed that palmitic acid (12.179%) and oleic acid (54.297%) were the major saturated and unsaturated fatty acids respectively. GLC analysis of mucilage hydrolysate showed that glucose (46.1%) was the major component while fructose (0.34%) was the minor component. GC/MS analysis of the essential oil the leaves of *Cotoneaster horizontalis* Decne proved that the hydrocarbons represented (3.82%), while oxygenated compounds represented (62.25%). Benzaldehyde (34%) was the major constituent of the total volatile oil composition. The mucilage content of the aerial parts of *Cotoneaster horizontalis* Decne exhibited both hypoglycemic and anti-dyslipidemic effects at an oral dose of 250 mg/kg body weight (b.wt., p.o.).
[Shaza A. Mohamed, Nadia M. Sokkar, Omyma El-Gindi, Zeinab Y. Ali, Iman M. Alfishawy. **Phytoconstituents Investigation, Anti-diabetic and Anti-dyslipidemic Activities of *Cotoneaster horizontalis* Decne Cultivated in Egypt.** *Life Sci J* 2012; 9 (2s):394-403]. (ISSN:1097-8135).http://www.lifesciencesite.com. 44

Keywords: *Cotoneaster horizontalis* Decne; Rosaceae; Anti-diabetic activity; Anti-dyslipidemic activity.

1. Introduction:

Family Rosaceae is a large family of flowering plants with about 95 genera and 2830 species; the name is derived from the type genus *Rosa* **(Potter, 2007)**. Among the largest genera in the family is the ornamentally important genus *Cotoneaster* with about 40 species of woody plants. The plant of interest in this genus is *Cotoneaster horizontalis* Decne which has the common name of Rockspray. *Cotoneaster horizontalis* Decne is semi evergreen, low shrub, its branches are spreading horizontally near the ground, the leaves are round to broadly elliptic, 0.25 to 0.5 inch long, dark glossy green , its flowers are pinkish to white colour , the fruits are bright red and it is wild in China **(Bailey, 1976)**.The aim of this study is to investigate polyphenols, flavonoidal, lipid, essential oil and mucilage contents of the aerial parts of *Cotoneaster horizontalis* Decne as well as to determine the anti-diabetic and the anti-dyslipidemic activities of its mucilage content.

2. Material and Methods:

Plant Material:

The aerial parts of *Cotoneaster horizontalis* Decne were obtained from EL - Orman botanical garden, Giza, Egypt. Voucher samples were kept in Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Standards and Reagents:

Solvents: acetonitrile , methanol, diethyl ether, *n*-hexane (HPLC grade, Merck); chemicals: Folin - Ciocalteu reagent (Merck), Barium hydroxide, sodium carbonate, aluminum tri-chloride, sodium acetate, sodium sulphate, potassium hydroxide, hydrochloric acid, sulphuric acid (analytical grade, BDH), acetic acid, phosphoric acid (HPLC grade, Merck); standard compounds: Gallic acid , ruin (Sigma Co., USA), streptozotocin (Sigma-Aldrich, USA), glibenclamide (DAONIL® 5 mg, Aventis Egypt). Biochemical kits [Spectrum Diagnostics (Hannover, Germany)]. Insulin kit [insulin enzyme-linked immunosorbent assay (ELISA)] (Dako Diagnostics, U.K.).

Animals:

Female Swiss strain albino rats (150 ± 20 g) and mice (20-25 g) were obtained from the animal house of National Organization for Drug Control and Research (NODCAR), Giza, Egypt. Animals were kept under standard laboratory conditions of light/dark cycle (12/12 hours), temperature (25 ± 2 °C) and fed on normal laboratory diet and water *ad libitium*. They were acclimatized for a week in the new environment before the experiment.

Colorimetric Estimation of Total Polyphenols:

The content of total polyphenolic compounds in methanolic extract of aerial parts of the plant under the study was determined by the method described in **(Folin and Ciocalteu, 1927)**. For the preparation of calibration curve 1ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanolic Gallic acid solutions were mixed with 5 ml Folin–Ciocaleu reagent (diluted ten-folds) and 4 ml (75g/l) sodium carbonate. The absorption was read after 30 min at 20 ^{o}C at 765 nm and the calibration curve was drawn. One ml plant extract (10 g/l) was mixed with the same reagents as described above, and after 1 hour the absorption was measured for the determination of plant polyphenols. All determinations were performed in triplicate. Total content of polyphenolic compounds in plant methanol extract was expressed as Gallic acid equivalent (GAE) and calculated by the following formula: [C = c. V/m], where: C= total content of polyphenolic compounds (mg/g) plant extract, as GAE; c= the concentration of Gallic acid established from the calibration curve (mg/ml); V and m= the volume and the weight of plant extract (ml) and (g).

Colorimetric Estimation of Flavonoids:

The content of flavonoids in aerial parts of the plant under the study was determined by a pharmacopeia method mentioned in **State Pharmacopeia of USSR (1989)** using rutin as a reference compound. One ml of plant extract in methanol (10 g/l) was mixed with 1 ml aluminum tri-chloride in ethanol (20 g/l) and diluted with ethanol to 25 ml. The absorption at 415 nm was read after 40 min at 20 °C. Blank samples were prepared from 1ml plant extract and 1 drop acetic acid, and diluted to 25 ml. The absorption of rutin solution was measured under the same conditions. Standard rutin solution was prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoids in plant extract was expressed as rutin equivalent (RE) and calculated by the following formula: $X = (A.m_0.10) / (A_0.m)$, where: X= flavonoids content (mg/g) plant extract as RE; A= the absorption of plant extract solution; A_0= the absorption of standard rutin solution; m= the weight of plant extract (g); m_0= the weight of rutin in the solution (g).

Colorimetric Estimation of flavonols content:

The content of flavonols compounds was determined by **(Yermakov *et al.*, 1987)**. The rutin calibration curve was prepared by mixing 2 ml of 0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml rutin ethanolic solutions with 2 ml (20g/l) aluminum tri-chloride and 6 ml (50 g/l) sodium acetate. The absorption at 440 nm was read after 2.5 hours at 20°C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of rutin solution. All determinations were carried out in duplicates. The content of flavonol compounds was expressed as rutin equivalent (RE) and calculated by the following formula: [X =C.V/m] where: X= flavonol content (mg/g) plant extract as RE; C= the concentration of rutin solution, established from the calibration curve (mg/ml); V and m= the volume and the weight of plant extract (ml) and (g).

HPLC Analysis of Total Polyphenols:

Extraction and purification of polyphenolic compounds from aerial parts of *Cotoneaster horizontalis* Decne were carried out using the procedure described in **(Goupy *et al.*, 1999)**. Separation and determination of polyphenols were performed by reverse phase HPLC (RP-HPLC)/diode array detection (DAD) (Hewlett Packard 110) using ODS RP C_{18} column, Hypersil (250 mm x4 mm i.d x 5 µm film thickness). The solvent system used was a gradient of A (CH_3COOH 8%) and B (acetonitrile) as follows : at 0 min, 5% A; at 20min, 10% A; at 50min, 30% A; at 55min, 50% A; at 60min, 100% A; at 100min, 50% A and 50%B; at110min, 100% B until 120min. The solvent flow rate was 1ml/min and separation was performed at 35°C. The volume injected was 10 µl for sample extract and 5 µl for the standards. Polyphenolic compounds were assayed by external standard calibration at 280nm and expressed in mg /100g dried plant powder.

HPLC Analysis of Total Flavonoids:

Extraction and hydrolysis of flavonoids from aerial parts of *Cotoneaster horizontalis* Decne were done according to the procedure mentioned in **(Mattila *et al.*, 2000)**. Separation and determination of flavonoids were performed by reverse phase HPLC (RP-HPLC)/diode array detection (Hewlett Packard 110) using ODS RPC_{18} column, Hypersil (250 mm x4 mm i.d x 5 µm film thickness). The solvent system used was a gradient of 50 mM H_3PO_4, pH 2.5 (solution A) and acetonitrile (solution B) as follows: Isocratic elution 95% A , 5% B, 0-5 min; linear gradient from 95% A, 5% B to 50% A, 50% B, 5-55 min; isocratic elution 50% A, 50% B, 55-65 min; linear gradient from 50% A, 50% B to 95% A, 5% B, 65-67 min. The solvent flow rate was 0.7ml/min and separation was performed at 35°C. Injection volume was 10 µl for sample extract and the standards. Flavonoids were

quantified using the external standard method at 270.4 nm for naringenin and hesperetin; 329.4 nm for luteolin and apigenin and 370.4 nm for myricetin, kaempferol, rhamnetin and quercetin, and the results were expressed in mg /100g dried plant powder.

Investigation of Lipid Contents:

Extraction of lipoidal matter:

Powdered air-dried aerial parts of *Cotoneaster horizontalis* Decne (150g) was extracted with petroleum ether (40-60°C) till exhaustion using soxhlet apparatus. Petroleum ether extract was evaporated to dryness under vacuum to give a residue of (1.4g).

Preparation of Un-saponifiable Matter:

The un-saponifiable matter was prepared adopting the procedure reported in **(Finar, 1973)**. One gram of the dried residue of the petroleum ether extract was saponified by reflux with 100 ml of 10% alcoholic potassium hydroxide for 5 hours. The liquid was distilled off almost to dryness. The residue was suspended in 100 ml water and extracted with diethyl ether till complete extraction of the un-saponifiable matter. The combined ethereal extract was washed with distilled water to remove any alkalinity, dried over anhydrous sodium sulphate and evaporated to dryness (0.4g).

Preparation of Fatty Acids Methyl Esters:

The alkaline aqueous layer left after extraction of un-saponifiable matter was acidified with dilute hydrochloric acid. The liberated fatty acids were extracted with successive portions of diethyl ether. The combined ethereal extract was washed with distilled water, dried over anhydrous sodium sulphate, evaporated to dryness (0.6g) and subjected to methylation using methanol and dry concentrated sulphuric acid **(Vogel, 1975)**.

GLC Analysis of Un-saponifiable Matter:

GLC analysis of un-saponifiable matter (2 µl) and standard material were carried out using HP 6890 with HP-5, Phenyl Methyl Siloxane column (30 m x 320 µm i.d x 0.25 µm film thickness) and flame ionization detector. The oven temperature was 70°C, injector temperature was 250°C and detector temperature was 300°C using temperature programming starting with 70°C for 2 minutes, increased to 280°C by the rate of 10°C/min., then isothermally for 30 minutes using nitrogen as a carrier gas with flow rate of 30 ml/min. Identification of constituents was carried out by comparison of their relative retention times with the available reference compounds. Quantification was based on peak area integration and internal normalization method.

GLC Analysis of Fatty Acids Methyl Esters (FAME):

GLC analysis of fatty acid methyl esters (FAME) (2 µl) and standard material were carried out using HP 6890 with flame ionization detector. The separation of fatty acids methyl esters was conducted using a Thermo TR-FAME, 70% Cyanopropyl Polysilphenylene Siloxane column (30 m x 0.25 mm i.d x 0.25 µm film thickness). The oven temperature was 140°C, injector temperature was 200°C and detector temperature was 220°C using temperature programming starting with 140°C for 3 minutes, increased to 200°C by the rate of 5°C/min., then isothermally for 25 minutes using nitrogen as a carrier gas with flow rate of 30 ml/min. Identification of constituents was carried out by comparison of their relative retention times with the available reference compounds. Quantification was based on peak area integration and internal normalization method.

Investigation of Mucilage Contents:

Extraction of Mucilage:

Fifty grams of air dried powder of aerial parts of *Cotoneaster horizontalis* Decne were soaked in 1 liter of acidulated water. The polysaccharides were precipitated from the aqueous extract by adding 4 volumes of ethanol 95% slowly while stirring, the precipitate obtained by filtration was purified, redissolved in hot water several times, treated with absolute ethanol, dried in desiccator until constant weight is obtained.1ml of 2% aqueous potassium hydroxide was added to 5ml of 1% aqueous solution of mucilage, the mixture was allowed to stand at room temperature for 15 min. No gel or semi gel or gelatinous precipitate was formed upon standing and on acidification with dilute acid, indicating that the precipitate is mucilage in nature **(Aboutabl *et al.*, 2008).**

Acid Hydrolysis of Mucilage:

Acid hydrolysis of mucilage was carried out as reported by **(Reda *et al.*, 1971)**. 0.1g of purified mucilage was refluxed with 10 ml of 1N HCL for 5 hours in boiling water bath then amount of Barium hydroxide was added followed by centrifugation. The precipitate was washed twice by water then evaporated until the volume reached to 2 ml.

GLC Analysis of the Mucilage Hydrolysate:

The mucilage hydrolysate (0.5 ml) was subjected to silylation according to the reported procedure in **(Kirk and Sawyer, 1991)** using a mixture of [tri-methyl chlorosilane and N, O-bis-trimethylsilyl acetamide, 1:5 by volume]. GLC analysis of mucilage hydrolysate (1µl) was performed, using HP 6890 instrument according the following conditions: Column ZB-1701, 14% Cyanopropyl Phenyl Methyl (30m x 0.25mm i.d x 0.25µm film thickness). The oven temperature was 150°C, injector temperature was 250°C and detector temperature was 270°C using temperature programming starting with 150°C for 2 minutes, increased to 200°C by the rate of 7°C/min., then isothermally for 20 minutes using helium as a

carrier gas with flow rate of 1.2 ml/min.Identification of the sugars was carried out by comparing the relative retention time of the peaks with those of the pure available authentic samples. The quantitative estimation of each peak was done by peak area measurement using a computing integrator.

Investigation of the Essential Oil Contents:

Extraction of Essential Oil:

Extraction of essential oil was done according to the method mentioned in **(Hamdan *et al.*, 2010)**. 200g of the fresh leaves of the plant under investigation was extracted with a mixture of *n*-hexane-diethyl ether (1: 1, v/v), filter then adding sufficient amount of methanol; the solvents were removed subsequently under a nitrogen stream and kept in a refrigerator for analysis.

GC/MS Analysis of Essential Oil:

The residue left after evaporation of solvents (1 mg) was dissolved in 1ml *n*-hexane and injected (1µl volume) into GC/MS equipped with MDS detector for analysis of volatile oil [HP 6890 and 5973 Series (Agilent) for GLC and mass analysis respectively] with capillary column TR-5MS, 5% Phenyl Polysilphenylene Siloxane (30m x 0.25 mm i.d x 0.25 µm film thickness). The oven temperature was 50°C, injector temperature was 250°C and detector temperature was 250°C using temperature programming starting with 50°C for 3 minutes, increased to 180°C by the rate of 5°C/min., then isothermally for 10 minutes using helium as a carrier gas with flow rate of 1ml/min. Identification of constituents was carried out based on MS fragmentation pattern referring to a computerized library program, Adams 2.0 (NIST, Gaithersburg, USA) as well as by comparing their retention times and mass spectra with published data in **(Adams, 1995)**.

***In Vivo* Anti-diabetic and Anti-dyslipidemic Activities:**

Preliminary Acute Toxicity Study:

Preliminary acute toxicity assessment was conducted according to the method described by **(Lorke, 1983).** Mice weighing 20-25 g b.wt., were separated into four groups of 6 mice each. They were fasted overnight and then were orally administered the mucilage at the doses of 300, 1000,2000,3000,4000 and 5000mg/kg b.wt. The incidence of mortality for each group was recorded up to 24 hours after administration. Food and water were provided *ad libitum*.

Experimental Design:

The experimental design reported by **(Said *et al.*, 2000)** was done. Diabetes was induced in rats by a single intra-peritoneal injection of streptozotocin (STZ) in a dose of 50 mg/kg body weight in freshly prepared citrate buffer (pH 4.5, 0.1 mole/l) injected in a volume of about 1 ml. The diabetic status of rats was assessed by measuring fasting blood glucose. Soon after streptozotocin injection, glucose water (5%) was given to rats for 2 days .A total of 40 rats were used, divided randomly into equal five groups (8 rats each) as follows; group 1: served as negative or normal control received only citrate buffer, group 2: normal rats treated daily with the mucilage (250 mg/kg b.wt.) ، group 3: untreated diabetic rats serve as positive control , group 4: STZ-induced diabetic rats treated with the mucilage (250 mg/kg b.wt), group 5: STZ-induced diabetic rats received glibenclamide (0.4 mg/kg b.wt.) as mentioned in **(Farook *et al.*, 2011)**. All groups had received their doses orally (p.o.), for a period of 4 weeks.

Oral Glucose Tolerance Test (OGTT):

At the end of the 28th day, 3 hours after the last dose of the mucilage (250 mg/kg b.wt., p.o) of the aerial parts of *Cotoneaster horizontalis* Decne or glibenclamide (0.4 mg/kg b.wt., p.o), blood samples were withdrawn from tail vein of overnight fasting rats and blood glucose was determined, indicating zero time of the test. Glucose solution (50%) in a dose of 2 g/kg b.wt., was given orally. Blood samples were withdrawn at 0, 30, 60, 90, and 120 min. after glucose loading and blood glucose level was determined at these time intervals using glucometer (ACCU-CHEK®, Roche, Switzerland). Curves of blood glucose levels (mg/dl) versus time intervals (min.) were constructed. The blood glucose concentration of each group were compared and tested for significance to the control diabetic (positive) group, to represent glucose utilization by the tissues.

Biochemical Analysis:

After four weeks, body weight gain, fasting blood glucose mentioned by **(Trinder, 1969),** glycosylated hemoglobin (HbA1c) (marker of evaluation of long term glycemic control and risk for chronic complications) reported by **(Trivelli *et al.*, 1971)**, serum insulin, serum lipid profile [triglycerides (TG), total cholesterol (TC) and high density lipoprotein-cholesterol (HDL)] and liver glycogen according to **(Carroll *et al.*, 1956)** were investigated. While low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were calculated by **Friedewald's equation (1972)** so, atherogenic risk factors (T.C/HDL & LDL/HDL) were calculated. Animals were weighed at the beginning and at the end of the experiment to detect the changes in their body weight gain. Serum insulin concentrations were determined by radioimmunoassay procedure using insulin kits.

Data Analysis:

The biochemical results are presented as mean ± S.E. and statistically analyzed using the Statistical Package for Social Sciences (SPSS, version 17; SPSS Inc, Chicago) for Windows followed by Dennett's

Multiple Comparison Test (DMCT). $P< 0.05$ was considered significant.

3. Results and Discussion:

Colorimetric Estimation of Total Polyphenols:

The content of phenolic compounds (mg/g) in methanolic extract was found to be 14 ± 0.16 mg/g plant extract and expressed as Gallic acid equivalent (GAE).

Colorimetric Estimation of Flavonoids and Flavonols: Flavonoids and flavonol contents were found to be 6.8 ± 0.76 and 2.2 ± 0.0 mg/g plant extract as rutin equivalent (RE) respectively.

HPLC Analysis of Total Polyphenols:

As represented in **Table 1**, HPLC analysis of the polyphenolic compounds showed the presence of eight identified compounds. Pyrogallol (1029.57 mg/100g dried plant powder) was the major component detected followed by chlorogenic acid (278.77 mg/100g dried plant powder) while caffeic acid was the least concentration compound (1.69 mg/100g dried plant powder).

Table 1. HPLC Analysis of Total Polyphenols of *Cotoneaster horizontalis* Decne Aerial Parts:

Identified Components	R_t (Retention time)	Concentration (mg/100g dried plant powder)
Pyrogallol	2.433	1029.57
Catechin	2.528	136.25
Chlorogenic acid	2.811	278.77
Caffeic acid	3.327	1.69
Vanillic acid	3.667	40.57
Syrengic acid	3.941	13.65
Salicylic acid	6.527	80.54
p-coumaric acid	6.724	10.50

HPLC Analysis of Total Flavonoids:

HPLC analysis of total flavonoids of *Cotoneaster horizontalis* Decne aerial parts revealed the presence of three identified compounds of different flavonoidal classes. Flavone class was represented by luteolin (9.2 mg/100g dried plant powder) which was the major flavonoid detected followed by the flavonol compound quercetin (3.41 mg/100g dried plant powder) and finally naringenin (0.38 mg/100g dried plant powder) which represented the flavanone class.

Investigation of Lipid Contents:

GLC Analysis of the Un-saponifiable Matter of *Cotoneaster horizontalis* Decne:

The percentage of the un-saponifiable matter was 40 % of the total lipid content in the plant. Sterols represent 28.4%, while hydrocarbons represent 46.81%. β-sitosterol (8.48%) was the major sterol followed by stigmasterol (1.09%) .α-Amyrine (14.35%) was the major triterpene and n-triacontane (17.11%) was the major hydrocarbon followed by n-hexacosane (13.52%) and n-henicosane (5.87%) in the un-saponifiable matter, as shown in **Table 2**.

Table 2. GLC analysis of the un-saponifiable Matter of *Cotoneaster horizontalis* Decne Aerial Parts:

Identified Components	RR_t*	Relative Area Percentage (%)
n-Pentadecane	0.448	0.80 %
n-Octadecane	0.558	0.86 %
n-Icosane	0.631	1.41 %
n-Henicosane	0.677	5.87 %
n-Docosane	0.697	1.70 %
n-Tetracosane	0.759	1.22 %
α-Amyrine	0.807	14.35 %
n-Hexacosane	0.829	13.52 %
n-Heptacosane	0.870	1.56 %
n-Octacosane	0.904	2.76 %
n-Triacontane	1	17.11 %
α-Tocopherol	1.069	4.48 %
Stigmasterol	1.104	1.09 %
β-Sitosterol	1.145	8.48 %
% of identified hydrocarbons		46.81%
% of identified sterols		28.4%

***RR_t = Relative retention times to the major hydrocarbon (n-triacontane) with R_t =27.215 min.**

GLC Analysis of the Fatty Acids Methyl Esters of *Cotoneaster horizontalis* Decne:

The percentage of the saponifiable matter was 60 % of the total lipid content in the plant under investigation. The major saturated fatty acid was palmitic acid which constitutes (12.18%) followed by myristic acid (7.02%) while the major unsaturated fatty acid was oleic acid which constitutes (54.3%) followed by linoleic acid (10.29%) as shown in **Table 3**.

Table 3. GLC Analysis of Fatty Acid Methyl Esters of *Cotoneaster horizontalis* Decne Aerial Parts:

Identified Components	RR_t*	Relative Area Percentage (%)
Methyl Caprylate	0.557	1.664 %
Methyl Caprate	0.597	2.659 %
Methyl Myristate	0.740	7.018 %
Methyl Palmitate	0.786	12.179 %
Methyl Stearate	0.968	2.664 %
Methyl Oleate	1	54.297 %
Methyl Linoleate	1.051	10.291%
Methyl Linolenate	1.115	4.122 %
Methyl Arachidate	1.279	3.297 %
% of saturated fatty acids		29.48%
% of unsaturated fatty acids		68.71%

***RR_t = Retention times relative to the major fatty acid (oleic acid) with R_t = 15.022 min.**

GLC Analysis of Mucilage Hydrolysate Contents:

A light brown colored powder was obtained after complete removal of moisture from the extracted mucilage. The yield of the mucilage obtained was 8 grams before hydrolysis. GLC study of mucilage hydrolysate revealed the presence of glucose (46.1%) as the major component followed by xylose (15%), arabinose (11%), L-rhamnose (5.9%), sorbitol (4.02%), mannitol (1.74%), galacturonic acid (1.72%), ribose (0.7%) and fructose was the minor component (0.34%) as shown in **(Table 4 and Figure1)**.

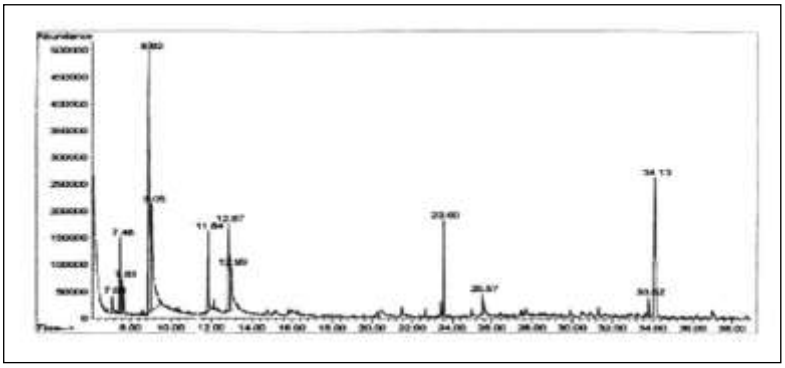

Figure 1. GLC Analysis Chromatogram of the Identified Sugars in Mucilage Hydrolysate of *Cotoneaster horizontalis* Decne Aerial Parts

Table 4. GLC Analysis of the Identified Sugars in the Mucilage Hydrolysate of *Cotoneaster horizontalis*, Decne Aerial parts:

Identified Components	RR_t*	Relative Area Percentage (%)
Xylose	0.682	15.69%
Arabinose	0.685	11.1%
Ribose	0.714	0.7%
Rhamnose	0.762	5.91%
Mannitol	0.886	1.74%
Sorbitol	0.898	4.02%
Fructose	0.915	0.35%
Glucose	1	46.16%
Galacturonic	1.109	1.72%

***RR_t=Retention times relative to the major component (glucose) with R_t =10.914 min.**

GC/MS Analysis of Essential Oil of *Cotoneaster horizontalis* Decne Leaves:

The essential oil isolated from the leaves of the plant under investigation comprises 15 identified components constituting (75 %) of the total oil composition. The total oxygenated compounds constituted the highest percentage of the components in the essential oil of the plant under investigation (62.25%) as seen in **Table 5**. GC/MS Analysis of the essential oil of the plant under investigation showed that benzaldehyde was the major constituent (34%) of the total essential oil composition as represented in **(Table 6 and Figure 2)**.

Table 5. Percentage of the Different Classes of Constituents in the Essential Oil of *Cotoneaster horizontalis* Decne Leaves:

Different classes of constituents in essential oil	Relative percentage (%)
Identified components	75 %
Unidentified components	25 %
Sesquiterpene hydrocarbons	0.65%
Total hydrocarbons	3.82 %
Oxygenated monoterpenes	4.89 %
Oxygenated sesquiterpenes	4.27 %
Total oxygenated compounds	62.25 %
Total monoterpenoids	4.89 %
Total sesquiterpenoids	4.92 %

Table 6. GC/MS Analysis of the Essential Oil Contents of *Cotoneaster horizontalis* Decne Leaves:

Peak No.	R_t (min.)	RR_t	Constituents of the Oil	Mass Spectral Data							M.W.	Area %
				M^+	B.P.	Major Peaks						
1	6.16	0.69	Unidentified	549	149	43	81	105	213	247	-	-
2	7.48	0.84	Unidentified	129	99	43	55	61	71	116	-	-
3	7.63	0.86	Dimethylhexane	114	43	50	57	71	85	99	114	2.00 %
4	8.89	1	Benzaldehyde	106	77	43	51	63	89	98	106	34 %
5	11.84	1.33	Dihydromyrcenol	156	59	43	55	67	83	95	156	4.37 %
6	12.15	1.37	Undecane	156	57	43	71	85	113	127	156	0.65 %
7	12.87	1.45	Methyl benzoate	136	105	39	51	77	91	119	136	6.23 %
8	15.81	1.78	Phlorone	136	39	43	79	91	108	121	136	0.18 %
9	20.44	2.30	Eugenol	164	40	77	91	121	131	149	164	0.52 %
10	21.51	2.42	Diphenyl ether	170	141	51	65	77	105	115	170	0.84 %
11	22.67	2.55	Unidentified	220	165	57	77	105	177	205	-	-
12	23.46	2.64	E,E-Alpha-Farnesene	204	93	55	69	79	107	119	204	0.65 %
13	23.60	2.65	Ionol (2,6-Di-tert-butyl-*p*-cresol	220	205	41	57	105	145	177	220	3.62 %
14	25.00	2.81	Unidentified	166	93	43	69	81	107	151	-	-
15	25.57	2.88	cis-3-hexenyl benzoate	204	105	51	67	77	82	123	204	1.04 %
16	27.73	3.12	Heptadecane	240	57	43	71	85	99	113	240	0.52 %
17	31.34	3.53	Pentadecanone	226	58	43	71	85	110	123	226	0.65
18	33.82	3.80	Unidentified	288	84	42	57	70	111	125	-	-
19	34.13	3.84	Methyl palmitate	270	74	43	55	87	143	227	270	9.97 %
20	37.00	4.16	Ethyl palmitate	284	88	55	101	115	157	241	284	0.83 %

*R_t = retention time, RR_t = Retention times relative to the major constituent (benzaldehyde) with R_t = 8.89 min., M^+ = molecular ion peak, B.P. = base peak, M. W. = molecular weight.

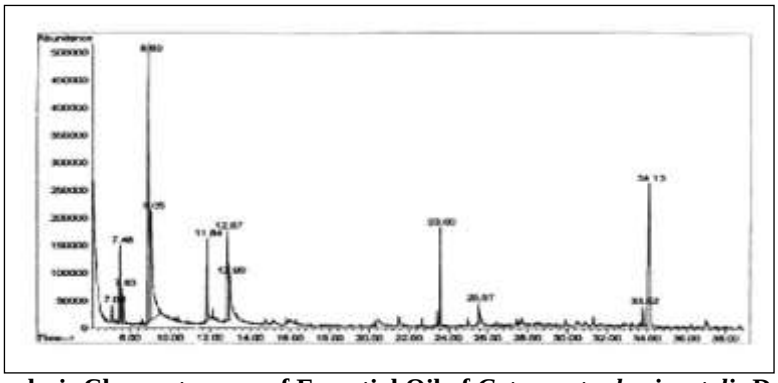

Figure 2. GC/MS Analysis Chromatogram of Essential Oil of *Cotoneaster horizontalis* Decne Leaves

Acute Toxicity Study:

The acute toxicity study done on the mucilage of the aerial parts of *Cotoneaster horizontalis* Decne showed no signs of toxicity up to 5 g / kg b.wt. and was considered as safe according to **OECD Guidelines (1996)**.One-twentieth of the maximum dose (250 mg / kg b.wt.) of the mucilage was selected for evaluation of anti-diabetic and anti-dyslipidemic activities.

***In Vivo* Anti-diabetic and Anti-dyslipidemic Activity:**

STZ injection resulted in diabetes mellitus close to that of humans, which is due to the destruction of *β*-cells of islets of Langerhans as seen in **(Table 7 and Figure 3)**. Acute insulin deficiency initially causes an increase in free fatty acid mobilization from adipose tissue. This results in an increased production of LDL-cholesterol particles **(Maiti *et al.*, 2004)**.

In diabetic (positive) control group, loss in body weight may be occurred as shown in **Table 8**, due to some abnormalities in carbohydrate metabolism such as lipolysis, glycogenolysis and acidosis, or it may be caused by disturbance in some metabolic pathways and results from protein deficiency **(Bhardwaj *et al.*, 2010)**. However, diabetic rats treated with the mucilage extracted from *Cotoneaster horizontalis* Decne aerial parts showed no significant change in body weight which is explainable by improvement in blood glucose level and increased food consumption as seen in **Table 8**.

Glycosylated Hemoglobin (HbA_{1c}) is a useful indicator of how well the blood glucose level has been controlled in the recent past and may be used to monitor the effects of diet, exercise and drug therapy on blood glucose in diabetic patients. Therefore, **WHO (2011)** concluded that HbA_{1c} can be used as a diagnostic test for diabetes. An HbA_{1c} of 6.5% is recommended as the cut point for diagnosing diabetes. In the present study positive control group had shown higher levels of HbA_{1c} (8.87 ± 0.21g/dl) compared to those in normal rats (5.81 ± 0.16g/dl) indicating their poor glycaemic control as represented in **Table 8**.

Increased non-enzymatic and auto-oxidative glycosylation is one of the possible mechanisms linking hyperglycaemia and vascular complications of diabetes **(Farook *et al.*, 2011)**.

Administration of the mucilage of the aerial parts of *Cotoneaster horizontalis* Decne at dose of (250 mg/kg b.w., p.o.) showed a marked improvement in glucose tolerance, body weight gain, blood glucose, glycated hemoglobin and liver glycogen, represented in **Table 8**, serum lipid profile and atherogenic risk factors, represented in **Table 9** in both normal and diabetic rats while serum insulin was insignificantly affected as shown in **Table 8**. Such an improvement effect may be accounted for a decrease in rate of intestinal glucose absorption and improvements in carbohydrate and lipid metabolism. There are a series of physical interactions in the gastrointestinal tract which affect the absorption, as water can be held within the polysaccharide matrix causing considerable swelling and viscous solution or gelation. Viscous polysaccharide gels may slow absorption by trapping nutrients, digestive enzymes or bile acids in the matrix and by slowing mixing and diffusion in the intestine **(Palanuvej *et al.*, 2009)**.

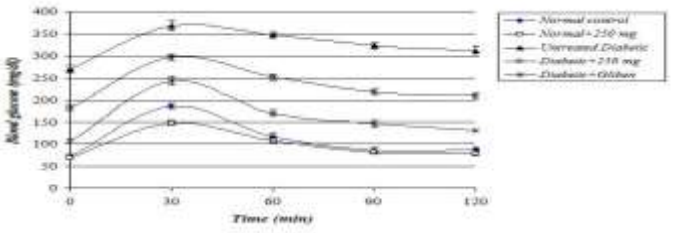

Figure 3. Glucose Tolerance Curves

Table 7. Oral Glucose Tolerance Test (OGTT):

Groups	Blood Glucose (mg/dl)				
	0 min.	30 min.	60 min.	90 min.	120 min.
1:Normal (negative) control	72.8 ± 4.42	185.8 ± 5.82***	116.5 ± 7.87***	84.8 ± 5.89ns	87.3 ± 4.70ns
2:Normal+ mucilage (250 mg/kg b.wt.)	76.9 ± 2.91	148.4 ± 5.46***	107.9 ± 5.22***	80.9 ± 3.25ns	78.0 ± 4.09ns
3:Untreated diabetic (positive) control	269.8 ± 8.22	368.4 ± 10.9***	347.1 ± 7.61***	323.0 ± 8.22***	311.5± 12.1**
4:Diabetic+ mucilage (250 mg/kg b.wt.)	181.4 ± 8.13	297.1 ± 5.75***	252.0 ± 7.15***	218.9 ± 5.39**	209.1 ± 7.56*
5: Diabetic + glibenclamide (0.4 mg/kg b.wt.)	105.4 ± 7.67	243.4 ± 10.3***	170.0 ± 7.34***	145.6 ± 8.38**	130.8 ± 8.01*

Each value represents the mean of 8 rats ± S.E.
ns: not significant at P >0.05, P^{*}<0.05; P^{**}<0.01; P^{***}<0.001; as compared with the corresponding zero time.

Table 8. Effect of Different Treatments on B.W Gain, Insulin, Glucose, HbA_{1c} and Liver Glycogen:

Groups	B.W gain (g)	S. Insulin (µU/ml)	Glucose (mg/dl)	HbA_{1c} (g/dl)	Glycogen (mg/g)
1:Normal (negative) control	45.6 ± 1.59^{c}	12.2 ± 0.14^{c}	80.1 ± 3.10^{a}	5.81 ± 0.16^{b}	38.4 ± 1.53^{c}
2:Normal + mucilage (250 mg/kg b.wt.)	39.1 ± 1.86^{b}	12.5 ± 0.22^{c}	68.9 ± 2.27^{a}	5.07 ± 0.11^{a}	40.1 ± 1.26^{a}
3:Untreated diabetic (positive) control	29.0 ± 1.75^{a}	8.98 ± 0.15^{a}	259.8 ± 4.83^{d}	8.87 ± 0.21^{d}	23.3 ± 1.46^{c}
4:Diabetic + mucilage (250 mg/kg b.wt.)	31.3 ± 1.37^{a}	9.01 ± 0.11^{a}	186.5 ± 6.84^{c}	7.89 ± 0.19^{c}	29.3 ± 1.57^{b}
5:Diabetic + glibenclamide (0.4 mg/kg b.wt.)	44.6 ± 1.90^{c}	11.5 ± 0.21^{b}	110.0 ± 12.3^{b}	6.26 ± 0.18^{b}	33.3 ± 1.94^{b}

Each value represents the mean of 8 rats ± S.E.
Values in the same column with the different superscript letters (a, b, c, d) are significantly different from each other at $P \leq 0.05$ level by DMCT.

Table 9. Effect of Different Treatments on Serum Lipid Profile:

Groups	T.G (mg/dl)	T.C	HDL	LDL	VLDL	T.C/HDL	LDL/HDL
1: Normal (negative) control	55.9 ± 5.38^{a}	96.0 ± 4.10^{a}	41.4 ± 2.01^{b}	43.4 ± 3.82^{b}	11.3 ± 1.07^{a}	2.11 ± 1.33^{a}	1.07 ± 0.11^{a}
2: Normal+ mucilage (250 mg/kg b.wt.)	47.0 ± 1.94^{a}	82.3 ± 2.96^{a}	45.1 ± 2.00^{b}	27.8 ± 1.37^{a}	9.41 ± 0.39^{a}	1.83 ± 0.03^{a}	0.62 ± 0.03^{a}
3:Untreated diabetic (positive) control	114.1 ± 4.90^{c}	181.9 ± 4.95^{c}	36.7 ± 1.27^{a}	122.4 ± 5.50^{e}	22.4 ± 0.86^{c}	5.01 ± 0.24^{c}	3.33 ± 0.23^{d}
4: Diabetic+ mucilage (250 mg/kg b.wt.)	88.2 ± 6.51^{b}	177.6 ± 8.02^{c}	38.7 ± 1.32^{a}	108.7 ± 5.18^{d}	17.2 ± 1.21^{b}	4.62 ± 0.26^{c}	2.81 ± 0.18^{c}
5: Diabetic+ glibenclamide (0.4 mg/kg b.wt.)	81.1 ± 6.06^{b}	145.0 ± 4.51^{b}	39.9 ± 1.70^{a}	88.9 ± 5.08^{c}	16.0 ± 1.18^{b}	3.69 ± 0.20^{b}	2.28 ± 1.87^{b}

Each value represents the mean of 8 rats ± S.E .
Values in the same column with the different superscript letters (a, b, c, d) are significantly different from each other at $P \leq 0.05$ level by DMCT.

Conclusion:

This study indicates that the aerial parts of *Cotoneaster horizontalis* Decne contain different classes of constituents as polyphenols and flavonoids. The investigation of lipid contents proved the presence of different hydrocarbons, phytosterols, saturated and unsaturated fatty acids. Also the plant leaves contain essential oil with high percentage of oxygenated compounds. The mucilage of the aerial parts of *Cotoneaster horizontalis* Decne at oral dose of 250 mg/kg b.wt. has both hypoglycemic and anti-dyslipidemic activities, which might be helpful in treating diabetic conditions associated with atherosclerosis or hyperlipidemia. The possible mechanism involved in these activities does not include the stimulation of insulin release from the remnant pancreatic *β*- cells but possibly involved by the physico-chemical characteristics of the mucilage.

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12/18/2012