

PURIFIED FRACTIONS OF *DODONAEA VISCOSA* SEEDS ON ANTIDIABETIC AND ANTIHYPERLIPIDEMIC ACTIVITIES ON RATS.

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Summary

Purified fractions: A₁ and A₄ of the plant *Dodonaea viscosa* (DV) seeds were prepared and investigated for its antidiabetic and antihyperlipidemic activities in albino rats. Butonal insoluble fraction of ethanolic extract of seeds of DV was subject to column chromatography and ethyl acetate: methanol fractions of A₁ and A₄ were used to screen the antidiabetic and antihyperlipidemic activities. Significant activities were observed for both the fractions (P<0.001). The findings suggest that fractions have antidiabetic and antihyperlipidemic effect by virtue of decreasing the levels of serum glucose and other lipid profiles.

Keywords: *Dodonaea viscosa*, antidiabetic, antihyperlipidemic activities, Insulin, Simvastatin.

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Introduction

There is still an unmet need for medicinal plants and phytopharmaceuticals with scientifically proven antidiabetic activity. Diabetes is a major health problem in developed and developing countries. Diabetes mellitus is characterized by a progressive decline in insulin action. The β -cells normally compensate insulin resistance by secreting more amount of insulin to maintain the glucose homeostasis. In non-insulin dependent diabetes mellitus this β -cells function gets impaired leading to deterioration in glucose homeostasis and subsequent development of impaired glucose tolerance and frank diabetes^{1,2}.

Hyperglycemia in the diabetics is associated with alteration of glucose and lipid metabolism and modification is liver enzymes level³. Liver is an important insulin dependent tissue which plays a vital role in glucose and lipid metabolism and is severely affected during diabetes⁴. Many of antidiabetic properties of plant products are also exhibits of lowering the lipid content. From the beginning of last century, evidence of lipid lowering properties of medicinal plants has accumulated⁵. Ethanobotanical information⁶ indicates that more than 800 plants are used as traditional and remedies for the treatment of diabetics and lowering the lipid content.

Dodonaea viscosa plant traditionally used to treat different human ailments including rheumatism, as febrifuse, antipruritic, hypotensive, antiviral and discutient⁷. They are also often used as neurological disorders⁸.

The aerial parts of the *Dodonaea viscosa* being used by ayurvedic physicians in the various parts of Karnataka for the treatment of diabetes. Hence the present study is focus to established antidiabetic and antihyperlipidemic activity of purified fractions of *Dodonaea viscosa*.

Materials and methods

Plant materials

Fresh dried seeds were procured during early winter season from young matured plants from Alagilawada, Davangere District, and Karnataka State. The plant was authenticated (voucher specimens:4/2004) by taxonomist of Botany Department of DRM Science College, Kuvempu University and Department of Pharmacognosy, Bapuji Pharmacy College, Davangere, India. Garbled seeds were procured, passed through sieve no.40 to get coarse powder and was used for studies.

Extraction and isolation

The air dried coarse powdered material was subjected to Soxhlet extraction successively by using solvents of increasing polarity namely petroleum ether (60-80°C), chloroform, ethanol and distilled water. All the extracts were evaporated to dryness by using rotary evaporator under reduced pressure and controlled temperature. The obtained ethanolic powder material was subjected for further purification and isolation. The isolation of pure compounds from the plant extracts was evaluated by Column chromatographic separation using silica gel of 100-200 mesh and 60-120 mesh.

Isolation of pure components

The isolation of pure components involved the following steps:

1. Chromatographic separation using silica gel (100-200 mesh)
2. Chromatographic separation using silica gel (60-120 mesh)

Chromatographic separation using silica gel (100-200 mesh)

The ethanolic extract (35 g) was stirred with n-butanol (500 ml) and left over night. The supernatant liquid was collected and the solvent was removed by using rotary flash evaporator, which yielded brown colored pasty mass (5 g) and it is labeled as n-butanol soluble fraction. The residue, which was obtained as grey powder was labeled as ethanolic fraction (n-butanol insoluble fraction).

The n-butanol insoluble fraction of ethanolic extract (20 g) was chromatographed over silica gel (100-200 mesh) on column 55 cm length and 6 cm diameter. Elution was carried out with solvent mixtures of increasing polarities. Fractions were collected in 100 ml portions and monitored by TLC (silica gel G as adsorbent, solvent system methanol: ammonia (200:3)) and the fractions showing similar spots are pooled together. Elution with ethyl acetate: methanol (EA: MeOH (40: 60) gave brown crystalline solid (1000 mg) and named as A₁. Similarly, elution with EA: MeOH (30:70) yielded shiny brown colored crystalline solid (1250 mg) and was named as A₂. Elution with EA: MeOH (20:80) yielded brown crystalline solid (530 mg) and designated as A₃. Elution with EA: MeOH (10:90) yielded light pale brown colored crystalline (350 mg) and named as A₄. Fraction A₅ was obtained upon elution with pure methanol (grey colored powder 80 mg).

Purification of A₁ and A₄ by column chromatographic separation using silica gel (60 – 120 mesh)

The A₁ and A₄ were found to be impure and it was contemplated to purify it further. Thus A₁ (1 g) and A₄ (350 mg) was chromatographed over silica gel (60-120 mesh) on column of 15 cm length and 3 cm diameter. Elution was carried out with solvents and solvent mixtures of increasing polarities. Fractions were collected (25 ml portions) and monitored on TLC (silica gel G as adsorbent, solvent system ethyl acetate:methanol 60:40 for A₁ and A₄ ethyl acetate:methanol 50:50) and the fractions showing similar spots were combined.

The last fraction A₁ yielded a light brown-crystalline solid showing a single spot on TLC with slight tailing. Whereas the last fraction of A₄ yielded a pale brown-crystalline solid showing a single spot on TLC. The fractions A₁ and A₄ showed the absence of nitrogen and sulphur in elemental analysis. This ruled out the possibility of the presence of any alkaloid or protein or any other nitrogenous compound. These fractions gave foam test and hemolytic test indicating the presence of saponin. They also showed the absence of steroid compounds as indicated by negative Libermann sterol test.

Characterization of pure compound

The pure compounds of A₁ and A₄ were characterized by different physical parameters and spectral studies.

Melting point

Melting point was recorded in an open capillary tube and it was found to be 221 to 225^o C (A₁) and 224 - 226^o C (A₄).

Ultraviolet spectrum

The UV spectrum was measured in aldehyde free spectral grade methanol using JASCO module UVIDEC-610 double beam spectrophotometer and UV-160A from Shimadzu Corporation, Japan.

Infrared spectrum

The infrared spectrums of the compounds are recorded in KBr in the range of 4600 to 400 cm⁻¹ on FTIR 5300 JASCO- (Research spectrometer series).

Nuclear magnetic resonance spectrum: ¹H NMR and ¹³C NMR

The nuclear magnetic resonance spectrum was recorded on SUPERCON-BRUK NMR spectrophotometer (200 MHz) using TMS as an internal standard. Sample was prepared by dissolving the compound in DMSO-d₆. Chemical shifts are expressed in δ ppm.

Mass spectrum

The mass spectrum was recorded on LCMS-2010A SHIMADZU.

Liquid Chromatography- Mass spectrum: The following method used in running LC-MS

HPLC-conditions:

Column-C₁₈

Mobile Phase : Methanol: Water mixture (90:10)

Flow rate : 0.2 ml/min

Volume injected : 5µl

Sample dissolved in methanol

Temp : 25°C

Probe : ESI (Electron Spray Ionization)
APCI (Atmospheric Pressure Chemical Ionization)

Experimental

Anti-diabetic activity^{9,10}

Acute toxicity study was performed using albino mice and doses were fixed as per OECD guideline No.420 and adopted CPCSEA protocol.

Wister rats were made diabetic by injecting alloxan monohydrate with a dose of 60mg/kg (i.p.), body weight is chilled citrate buffer (pH 4.5), after 8 hours, the rats showing blood glucose level of 250-350 mg/dl were considered as diabetic were employed in the study. The study was carried out following the guidelines of principles of laboratory animal care.

Group I : Served as solvent control.

Group II : Served as diabetic control (alloxan-induced)

Group III : Received fraction- A₁ (30 mg/kg, p.o)

Group IV : Received fraction-A₄ (30 mg/kg, p.o) and

Group V : Received insulin (0.6 /Kg, sc.)

All the test samples were administered daily and treatment period for all groups were two weeks on the 0-week, 1st and 2nd weeks, the animals were tested for 8 hr and blood samples were drawn by orbital sinus puncture under mild anaesthesia. The blood samples were collected in Eppendorf's tube that contained 50µl of EDTA (anti-coagulant). The determination of serum glucose was performed by enzymatic method (GOD/POD-method-Beacon-Diagnostic Pvt Limited, India).

Statistical Analysis: Data were statically analyzed as meant ±SEM and expressed as non significant P<0.001 using one way ANOVA followed by Dunnett's test.

Anti-hyperlipidemic activity

Anti-hyperlipidemic activity of fractions A₁ and A₄ was performed on Triton-induced hyperlipidemic rats and was evaluated as per the method described by Moss¹¹, Vogel¹² and Hirsch. Animals were divided into five groups.

Group I : Served as solvent control.

Group II : Served as hyperlipidemic control (Triton-induced) 400 mg/kg., i.p.)

Group III : Received fraction-A₁ (30 mg/kg., p.o.)

Group IV : Received fraction-A₄ (30 mg/kg., p.o.) and

Group V : Received Simvastatin (10 mg/kg., i.p.)

After 18 hours blood samples were collected from retro orbital sinus puncture under mild anesthesia and transferred directly into centrifuge tube and allowed to clot at room temperature for 20-25 min and centrifuged at 2000 rpm. The supernatant clear serum thus obtained was transferred carefully with the help of micropipette into small test tubes for estimation. The serum concentration of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol were measured by standard procedure using auto analyzer (RA 50 model, Miles India Limited).

Statistical Analysis

Data were statically analyzed as meant ±SEM and expressed as non significant P<0.001 using one way ANOVA followed by Dunnett's test.

Results and Conclusions

The purified fractions of A₁ and A₄ of DV has been isolated by employing various techniques and identified on the basis of spectral studies.

The result of the present study demonstrates the efficiency of fractions of DV is lowering the serum glucose level in alloxan induced diabetic rat models. Similarly lowering the serum cholesterol, triglycerides in Triton-induced hyperlipidemic rats. Secondary plant metabolites such as flavonoids, saponins terpenoids may be responsible for the antihyperlipidemic activity. The triterpenoids and flavonoids from DV may augment the activity of lecithin acyl transferase (LCAT), which regulates blood lipids and glucose concentrations. The LCAT plays a key role in the incorporation of free cholesterol into HDL (this may increase HDL) and transferring it back to VLDL and LDL which are taken back later in liver cells. Several investigation reports have positive witnessed that, increase in HDL-C is associated^{13,14,15} with decrease in coronary artery diseases.

However, contribution of antidiabetic and hypolipidemic activities could be due to purified fractions of *Dodonaea viscosa* seeds.

Table-1: Antidiabetic activity of fractions of *Dodonaea viscosa* seeds

Treatment	Serum glucose mg/dl				
	0 week	1 st week	% reduction	2 nd week	% reduction
I Tween-80(1%)	80.6±0.40	81±0.31	0.4	80.6±0.40	---
II Diabetic control (60 mg/kg.)	255.0±2.2	285±2.2	11.8	331.7±7.5	-30
III Diabetic control + Insulin (0.6U/kg)	258.3±3.1	154.2±0.2	40.3	119.2±2.0	54
IV Diabetic control + A ₁ (30 mg/kg.)	251.8±1.11	168.8±0.37	33.0	130.6±0.40	48.13
IV Diabetic control + A ₄ (30 mg/kg.)	261.2±0.73	199.6±0.50	23.58	187.0±0.96	28.42

Oneway ANOVA, values are SEM : n = 6 in each group
P < 0.001 as compared to diabetic control

Table-2: Estimation of Serum Parameters in Triton-induced hyperlipidemic rats.

Treatment groups	Lipids Profile			
	Total cholesterol	Triglycerides (TG)	HDL-cholesterol	LDL-cholesterol
I Control (Tween-80,1%)	35.5±0.64	52.05±1.19	20.75±0.47	25.25±0.47
II Triton (400 mg/kg)	97.75±1.31	82.75±1.60	21.75±1.18	85.75±2.07
III Triton + A ₁ (30 mg/kg)	62.75±1.10	53.75±1.31	41.75±1.18	53.75±1.31
IV Triton + A ₄ (30 mg/kg)	52.75±1.60	46.5±2.21	44.75±0.47	53.75±1.31
V Triton + Simvastatin (10 mg/kg)	41.75±0.85	53.25±1.10	26.25±1.31	28.25±1.65

One way ANOVA followed by Dunnett's test p<0.01 NS p<0.001 HS

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