

Antihyperlipidemic Activities of Isolated bio Compounds of Aegle Marmelos

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Abstract: The present work was done to study qualitative investigation of extractability of the useful compounds from *Aeglemarmelos* leaves via supercritical fluid extraction technique (SFE) and to evaluate antihyperlipidemic activity of *Aeglemarmelos*(Am). The obtained extract and fractions were treated on Triton induced rat models and the results showed lowering the lipid profile level in the experimental models by in-vivo methods. The fractions of *Aeglemarmelos*(AmF1) also exhibited excellent anti hyperlipidemic property at 3rd week and also lowering the serum cholesterol, triglycerides in Triton induced rats. The most important ingredients present in plant communities turn out to be alkaloids, terpenoids, steroids, phenols glycosides and tannins are responsible for monitoring the normal lipid profile in Triton induced rats due to LA of lecithin acetyl transferase (LCAT), which regulates lipids concentration. 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. The changes in the level of serum lipids in experimental rats are illustrated in (Table 1). The total Cholesterol, TG1, LDL and VLDC significantly decreased ($P < 0.001$) and simultaneously there is increase in level of HDLs. Supercritical Fluid extraction of leaves of *Aeglemarmelos*(Am) possess significant antihyperlipidemic activity as compared to control group.

Keywords: *Aeglemarmelos*, Triton, Lipid profile, supercritical fluid extraction, Column chromatography

I. Introduction

Hyperlipidaemia is the greatest risk factor contributing to atherosclerosis [1-5] and occurrence of coronary heart disease and cerebrovascular accidents. Hence hypolipidemic [6] molecules are extensively used as prophylactic agents to prevent such atherosclerosis induced disorders. But these hypolipidemic drugs [7] are not free from adverse effects. Pancreatitis due to moiety of statin is a well-known adverse effect and HMG-CoA reductase inhibitors have also been reported to cause hyperglycemia and FDA has given a warning to this effect. Many plant derivatives and domestic remedies have been screened for their hypolipidemic action. More than 70 medicinal plants have been documented to have significant hypolipidemic action. Beginning of last century evidence of lipid lowering [8] properties of medicinal plants has accumulated [9-11]. Ethno botanical information [12] indicates that more than 800 plants are used as traditional and remedies for the treatment of diabetes and lowering the lipid profile. Taking these findings forward is mandatory to develop new drugs in this area. Hence further research into identifying the active principle, conducting preclinical studies and if possible clinical studies is needed to evaluate the Antihyperlipidemic [13-16] activities of this plant.

II. Materials And Methods

Plant

Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds. Plants are known to be the source of many chemical compounds. *Aeglemarmelos* [17-29], commonly known as Bael, is a spiny tree belonging to the family Rutaceae Sub family: Aurantioideae Genus: *Aegle*. The leaves, roots, bark, seeds and fruits are edible and have medicinal values. The plant was authenticated by taxonomist and specimen (No.24/Am) is preserved in the department of Research Pharmacology, Bapuji Pharmacy College, Davangere (India).

2.1 Supercritical extraction³⁰

The leaves of *Aeglemarmelos* as raw material were collected from a home garden. The leaves collected were washed with tap water to remove the dust and dirt. They were dried under sun drying for 3 hours. The dried leaves were powdered using a mixer to obtain powder of the raw leaves of *Aeglemarmelos*. Carbon dioxide (purity 99.99%) as solvent contained in a dip tube cylinder is installed. Supercritical CO₂ was obtained by using SFT-10 Supercritical Fluid Pump. The SFT-10 is a high precision carbon dioxide pump designed to deliver liquid carbon dioxide at pressures up to 10,000 psi (68.9 MPa).

2.2 Experimental procedure

1. Turn on the power to the SFT-110 SFE unit.
2. Turn on the Peltier cooler at least thirty minutes prior to the start of the experiment.
3. Powder 50 grams of raw material i.e., dried *Aeglemarmelos* leaves.
4. Place the powdered *Aeglemarmelos* leaves into the SFT-110 Unit's processing vessel and seal.
5. Ensure that both the static/dynamic valve and restrictor valve are closed.
6. Open the carbon dioxide tank valve to allow the carbon dioxide to come into the unit (~750 psi).
7. Set the oven temperature to 40°C and the restrictor block to 40°C.
8. Set the pressure on the pump to 2000 psi. The pump should begin to actuate to pressurize the sample vessel. This will take between 12-15 minutes.
9. Once the pressure is up to 2000 psi, allow the sample to "soak" at that pressure for 15 minutes for 40°C.
10. Open the static/dynamic valve to allow free flow of carbon dioxide through the restrictor valve. Adjust the restrictor valve to achieve about 24ml/min of liquid carbon dioxide. Flow dynamically for 15 minutes. The pump should actuate and continue to maintain sample vessel pressure.
11. Close the static/dynamic valve and allow to "soaking" for an additional 15 minutes before repeating the dynamic flow step above. Repeat the static soak and dynamic flow step 5 more times.
12. Set the vessel temperature to ambient and set the pressure control down to ambient. Allow the unit to vent. When the vessel has reached ambient pressure, disconnect the inlet and outlet fittings, open the vessel and remove the *Aeglemarmelos* powder.
13. Similar procedure is carried out by varying the parameters such as temperature (45°C, 50°C and 55°C), pressure (2000 psi, 3000 psi and 4000 psi) and time (20, 25 and 40 minutes).
14. Phytochemical investigations of all extracts were carried out in order to detect the presence of the following class of compounds.

2.3 Column chromatography

The obtained *Aeglemarmelos* powder (10 gm) was subjected to column chromatographic methods. The supercritical fluid extract (10 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 adsorbent) and the fractions showing similar spots are pooled together. Elution with ethyl acetate: ethanol (EA: ET-OH (40: 60) gave brown crystalline solid (450 mg) and named as AmF1. Similarly, elution with EA: ET-OH (30:70) yielded shiny brown coloured crystalline solid (1.5g) and was named as AmF2.

2.4 Acute toxicology test³¹

Acute toxicity study was performed using albino mice and doses were fixed as per OECD guideline No.423 and adopted CPCSEA protocol. Wister rats were made hyperlipidemic by injecting Triton-induced with a dose of 400 mg/kg (i.p), body weight is chilled citrate buffer (pH 4.5),

2.5 Anti-hyperlipidemic activity

Anti-hyperlipidemic activity of fractions AmF1 and AmF2 was performed on Triton-induced hyperlipidemic rats and was evaluated as per the method described by Moss¹¹, Vogel¹² and Hirsch. Animals were divided in to six groups.

Group I: Served as solvent control.

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

Group III: Received Statins (5mg/Kg, p.o)

Group IV: Received extract (150 mg/kg, p.o)

Group V: Received extract (75 mg/Kg, p.o)

Group VI: Received fraction-1 (AmF1-75 mg/kg, p.o).

After 18 hours blood samples were collected from retro orbital sinus puncture under mild anaesthesia 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 analyser (RA 50 model, Miles India Limited).

2.6 Statistical Analysis:

Data were statically analyzed as meant \pm SEM and expressed as significant $P < 0.001$ using one way ANOVA

followed by Dunnett’s test.

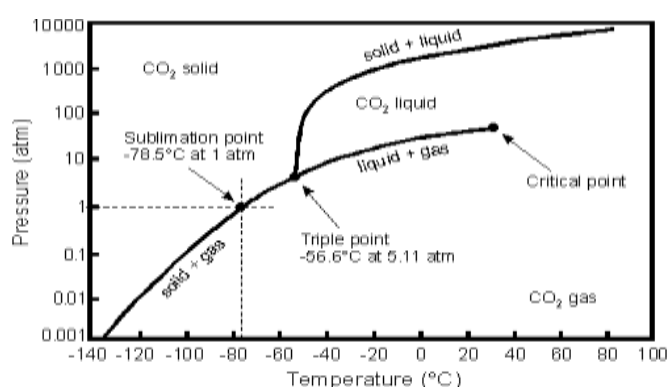
III. Results And Discussion

The present investigation was focused on to evaluate antihyper-lipidemic activities of *Aeglemarmelos* leaves extract and its fraction AmF1. The result of the present study demonstrates the efficiency of Supercritical extract Am and fraction AmF1. The plant secondary metabolite like flavonoids are responsible to monitoring the normal lipid profile is Triton induced diabetic rats due to LA of lecithin acetyl transferase (LCAT), which regulates blood lipids and glucose concentration. 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 with decrease in coronary artery diseases. The result of the present study suggested that extract and fraction of (AmF1) illustrate significant - antihypolipidemic activity ($P < 0.001$ HS) and results were tabulated in Table - 1.

IV. Figures And Tables

4.1.1 Figure (1)



Pressure-Temperature phase diagram for CO₂.

Fig. 1 Phase diagram for CO₂

4.1.2 Figure (2) Supercritical extraction set up.

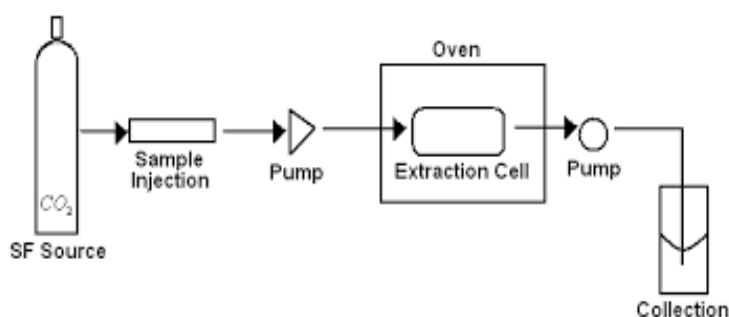


Fig. 2 Schematic diagram of Supercritical extraction set up.

3.2 Tables

Table 1 Estimation of serum parameters in Triton induced Hyperlipidemic rats.

Group	TC	TG	HDL	LDL	VLDL
Group I	100.79±24.53	90.25±35.95	35.83±3.09	65.00±38.54	30.50±5.49
Group II	105.33±33.09	94.29±39.79	35.75±3.99	70.21±39.02	33.29±6.33
Group III	104.34±30.69	92.70±40.36	34.65±4.57	67.17±37.59	32.13±5.10
Group IV	103.17±29.39*	91.70±37.01*	34.04±3.67*	65.78±36.00*	32.35±4.22*
Group V	104.35±31.28*	91.52±38.07*	36.30±3.92	65.83±37.03*	31.30±4.52*
Group VI	106.22±29.27*	91.30±38.37*	34.52±3.55	66.48±37.57*	31.39±4.73*

One way ANOVA followed by Dunnett’s test *Indicates difference from groups = $p \leq 0.001$ HS.

V. Conclusion

The present investigation was focused on to evaluate antihyper-lipidemic activities of leaves extract

of *Aeglemarmelos* and its fractions. The result of the present study demonstrates the efficiency and fractions of the Supercritical Fluid extracts of *Aeglemarmelos*(*Am*) in lowering the serum cholesterol, triglycerides in rats. Secondary plant metabolites such as flavonoids, saponins, terpenoids, and alkaloids may be responsible for the antihyperlipidemic activity.

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