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EVALUATION OF ANTIOXIDANT AND HYPOLIPIDAEMIC POTENTIAL OF HERBAL LEAF EXTRACT

Neeraj Jain*, Neelam Jain[1], Divya Dwivedi[2], Laxmi Mishra[3]

*Teerthankar Mahaveer College of Pharmacy, TMU, Moradabad- 244001, U.P., India.
[1]Faculty of Pharmacy, Oriental University, Indore-453555, Madhya Pradesh, India.
[2]Sakshi College of Pharmacy, Kanpur- 208017, U.P., India.

[3]Faculty of Pharmaceutical sciences Rama University, Mandhana, Kanpur-209217, U.P., India.

***Corresponding author name:** Neeraj Jain
Address: Teerthankar Mahaveer College of Pharmacy, TMU, Moradabad- 244001, U.P., India.
Email id: jneerajdops@gmail.com
Contact no: +91 8462933618

ABSTRACT

Objective: The present study was aimed to evaluate the leaves of *Elaeocarpus ganitrus* for its antioxidant and hypolipidaemic potential.

Methods: E. ganitrus leaf powder was extracted in ethanol and the crude extract was divided into aqueous ethyl acetate, butanol, methanol and 70:30 (ethanol: water) mixture yielded pure compounds like gallic acid, quercetin and ellagic acid. Ethanolic extracts of leaves were subjected to phytochemical screening to determine the presence of alkaloids, steroids, saponins, glycosides and flavonoids. Free radical scavenging techniques (DPPH and ABTS) and the ferric reducing antioxidant power (FRAP) were used to measure the antioxidant activity. Diet-induced hyperlipidemic model and triton-induced hyperlipidaemic model was used to measure the hypolipidaemic potential.

Results: Based on the findings, it was concluded that *E. ganitrus* is a very plant in several components such as flavonoids, triterpenes, quercetin and carbohydrates. The active fraction of *E. ganitrus* leaves showed potent antioxidant activity against DPPH free radical. Triton-induced hyperlipidemic control rats had significantly higher serum lipid and lipoprotein levels. Simultaneously, a rise in HDL-cholesterol was noted. When compared to hyperlipidemic controls, *E. ganitrus* leaves chloroform extract significantly lowered blood lipids ($p<0.001$).

Conclusion: Thus, the leaves of *E. ganitrus* may be considered as a promising plant extract for the control of oxidation and hyperlipidaemia.

KEYWORDS

E. ganitrus leaves; Hypolipidemia; Anti-oxidant; Cholesterol; DPPH.

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INTRODUCTION

Oxidative stress is strongly related to atherogenesis. An antioxidant which inhibits oxidation of LDL should be effective for suppressing atherosclerosis. Antioxidants have the ability to protect the body from oxidative damage by scavenging the free radicals and inhibiting peroxidation and other radical mediated processes [1]. Hyperlipidaemia (mainly increased level of total cholesterol (TC), triglycerides (TG) and lowdensity lipoprotein (LDL) cholesterol along with decrease in high-density lipoprotein (HDL) cholesterol contributes significantly to the manifestation and development of atherosclerosis and coronary heart diseases (CHDs). Natural plants have a dramatic cholesterol lowering properties without any side effects which are normally associated currently available hypolipidemic drugs. The consumption of synthetic hypolipidemic drugs having adverse effects like hyperuricemia, diarrhea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function. Medicinal plants play a major role in mitigating the atherosclerosis associated with oxidative stress and suggest that the lipid lowering action is mediated through inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine [2].

Elaeocarpus ganitrus (Family-Elaeocarpaceae) is commonly known as Rudraksha is a large evergreen broad-leaved tree, prevalent for its fascinating fruit stones and medicinal properties [3]. It contains various phytoconstituents which have pharmacological activities like antiinflammatory, analgesic and sedative, hypoglycemic, antidepressant, antiasthmatic and antiulcerogenic, antihypertensive, anticonvulsant and antimicrobial. Therefore, in the present study, the leaves of *E. ganitrus* has been assessed for its antioxidant and hypolipidaemic potential [4].

MATERIALS AND METHODS

The isolation of active chemical constituent of *Eleocarpus ganitrus* leaves was done by collecting them from a cultivated source, Hind Herb Store, Saharanpur, Uttar Pradesh, India. Methanol, ethyl acetate, hexane, chloroform, and butanol (Dramstadt, Germany) was obtained from Merck Laboratories Pvt. Ltd. (Mumbai, India). Charcoal and sodium sulphate were purchased from procured from Loba Chemie Pvt. Ltd. (Mumbai, India). Size exclusion chromatography was done using Sephadex LH-20 (Ameshem Bioscience), which was pre-swollen in the designated solvent before loading on the column. Vacuum liquid chromatography was done using silica gel 60GF254 and flash column chromatography was done using silica gel 60 (E. Merck 1.09385, Model Aldrich).

Extraction and fractionation

E. ganitrus leaf powder was extracted in ethanol and the crude extract was divided into aqueous, ethyl acetate, butanol, methanol and 70:30 (ethanol: water) mixture. The EA phase was dried with sodium sulphate, charcoaled and evaporated in vacuum to yield a thick residue that was subjected to normal pressure column chromatography on gradient system with hexane, chloroform and methanol yielding pure compounds gallic acid, quercetin and ellagic acid [5, 6].

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Determination of antioxidant activity

DPPH scavenging activity

The reduction in DPPH absorption was used to assess a substance antiradical potency (1, 1-Diphenyl-2-Picrylhydrazyl). 50 ml of the plant extract was mixed with 950 ml of a 0.1 mM DPPH solution. The absorbance of the combination was measured at 365 nm after 30 minutes. The following formula was used to calculate the ability to scavenge DPPH radicals [7]:

$$\% \text{ Inhibition of DPPH} = Ac\text{-}As/Ac \text{ X } 100$$

Where,

Ac = Absorbance of control

As = Absorbance of sample

Determination of antihyperlipidemic activity

Extraction of plant material

The leaves were mechanically ground coarsely before being extracted using the soxhlet method for 48 hours with 70% ethanol. The ethanol was then filtered. After being separated in a rotary evaporator under low pressure and a controlled temperature (55–60 C), the solid material was then kept in a desiccator. The plant's petroleum ether (60–80 °C), chloroform, alcoholic, and aqueous extracts each included 500 mg/kg of tween–80 as a suspending agent. The dose administered determined the suspension's strength, which was represented as the weight of dried extract [8].

Animal model

Adult wistar rats (150–200 g) of either sex were obtained and kept in cycles of 12 hours of light and 12 hours of darkness. For the experiment's baseline diet, standard pellets were used. Unrestricted access to food and water was given to both the experimental and control animals [9]. All animal research were carried out in accordance with the moral guidelines established by the institutional ethics council IAEC, the CPCSEA, and the Ministry of Social Justice and Empowerment of the Government of India.

Preparation of standard drug

Simvastatin 10 mg/kg was used as the reference standard drug to measure the antihyperlipidemic effect, and tween-80 was used to suspend it in distilled water.

Triton-induced hyperlipidaemic model

After fasting for 24 hours, animals were given a 400 mg/kg intra-peritoneal injection of triton saline solution. With the first dosage administered immediately following the triton injection and the second dose administered 20 hours later, the plant extracts were administered orally by stomach intubation at a dose of 500 mg/kg. After 4 hours from the second treatment, the animals were tested for several biochemical markers. Serum was obtained by centrifuging blood for 30 minutes at 2000 rpm from the rat's orbital plexus while the animal was under ether anaesthesia.

Experimental Design

There were seven groups of six creatures each created from the animals. Group I served as the conventional control group, receiving only the standard regular pellet diet and no triton

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diet. Simvastatin (10 mg/kg/day p.o.), a common antihyperlipidemic drug, was administered to Group II as a positive control. The hyperlipidemic control group, Group III, was given just an triton diet for hyperlipidaemia brought on by triton. E. ganitrus leaf extracts (500 mg/kg/day, p.o.) were given to groups IV, V, VI, and VII. These groups received treatment for triton-induced hyperlipidemia for 48 hours and 14 days, respectively. Under mild ether anaesthesia, blood was collected by a retro-orbital sinus puncture. The acquired samples underwent a 10-minute centrifugation.

Biochemical analysis

Using a traditional approach, the serum was examined for total cholesterol, triglycerides, phospholipids, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) levels (VLDL).

RESULTS AND DISCUSSION

Table 1 displays the antioxidant activity as measured by DPPH approach. For the plant leaves, the IC50 for DPPH radical scavenging was 132.6±10.07 µg/ml. *E. ganitrus* is a highly rich plant containing gallic acid, quercetin and ellagic acid which have the potential to serve as a reducing initiator by preventing oxidative reactions triggered by singlet oxygen.

Table 1: Antioxidant activity of *E. ganitrus* leaves extract assessed by DPPH method

Activity	DPPH (%)	IC50 (µg/ml)
E. ganitrus leaves	80.53±0.68	132.6±10.07

Trion-induced hyperlipidemic model

The hyperlipidemic control group that received treatments with aqueous, alcoholic, chloroform, and petroleum ether extracts of *E. ganitrus* leaves was treated with triton WR1339, which raised blood lipid and lipoprotein levels. These levels were maintained throughout the study. The results were comparable to those achieved with simvastatin, the industry standard. In comparison to normal control rats, triton-induced hyperlipidemic control rats had significantly higher serum lipid and lipoprotein levels. Simultaneously, a rise in HDL-cholesterol was noted. When compared to hyperlipidemic controls, E. ganitrus leaves chloroform extract significantly lowered blood lipids as shown in Table 2 and Table 3.

Table 2: Effect of *E. ganitrus* leaves extracts on serum cholesterol, triglycerides and phospholipids level in triton induced hyperlipidemic rats

Group Name* (Dose)	Values are expressed as mg/dL, Mean±SEM								
	Cholesterol			Triglycerides			Phospholipids		
	6hr	24hr	48hr	6hr	24hr	48hr	6hr	24hr	48hr
Normal control (vehicle only)	60.23± 0.32	65.12± 0.72	63.43± 0.52	65.23± 0.62	68.43± 1.32	62.53± 1.22	73.43± 1.22	75.13± 0.62	74.03± 1.62
Hyperlipidemic Control	260.23 ±2.02	176.20 ±2.72	102.33 ±1.54	209.56 ±3.62	106.23 ±2.43	105.12 ±1.34	107.65 ±5.45	183.13 ±0.72	101.13 ±5.62

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Simvastatin (10mg/kg)	84.63±2.32	165.12±5.72	73.43±2.52	85.30±1.62	168.43±1.32	78.33±2.22	91.43±1.34	135.23±1.60	100.03±3.62
Chloroform extract (500mg/kg)	85.13±2.32	191.32±3.72	91.63±3.52	86.63±5.62	184.33±3.32	83.03±1.56	93.43±4.23	155.63±4.62	84.63±2.62
Alcoholic extract (500mg/kg)	83.53±1.42	195.62±5.72	89.23±3.53	87.23±7.42	178.14±5.32	85.53±1.22	93.23±3.12	155.13±3.62	85.03±2.62
Aqueous extract (500mg/kg)	93.23±3.32	225.12±0.72	135.43±0.52	65.23±0.62	68.43±1.32	62.53±1.22	73.43±1.22	75.13±0.62	74.03±1.62
Petroleum ether extract (500mg/kg)	103.23±2.72	255.12±5.72	174.43±2.52	209.56±3.62	106.23±2.43	105.12±1.34	107.65±5.45	183.13±0.72	101.13±5.62

Table 3: Effect of *E. ganitrus* leaves extracts on LDL, VLDL and HDL level in triton induced hyperlipidemic rats

Group Name* (Dose)	**Values are expressed as mg/dL, Mean±SEM**								
	LDL			**VLDL**			**HDL**		
	6hr	**24hr**	**48hr**	**6hr**	**24hr**	**48hr**	**6hr**	**24hr**	**48hr**
Normal control (vehicle only)	55.23±1.32	57.12±0.72	61.43±1.52	25.23±0.62	18.43±1.32	15.53±1.22	33.43±1.22	35.13±0.62	44.03±1.62
Hyperlipidemic Control	103.23±2.02	196.20±2.72	95.33±1.54	29.56±3.62	36.23±2.43	35.12±1.34	17.65±5.45	18.13±0.72	21.13±5.62
Simvastatin (10mg/kg)	64.63±2.32	145.12±5.72	66.43±2.52	25.30±1.62	26.43±1.32	18.33±2.22	31.43±1.34	25.23±1.60	41.03±3.62
Chloroform extract	85.13±2.32	165.32±3.72	81.63±3.52	23.63±5.62	28.33±3.32	23.03±1.56	28.43±4.23	25.63±4.62	34.63±2.62

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(500mg/kg)									
Ethanolic extract (500mg/kg)	90.53± 1.42	175.62 ±5.72	85.23± 3.53	27.23± 7.42	32.14± 5.32	25.53± 1.22	26.23± 3.12	22.13± 3.62	31.03± 2.62
Aqueous extract (500mg/kg)	93.23± 3.32	185.12 ±0.72	95.43± 0.52	29.23± 1.62	35.43± 1.32	28.53± 1.22	22.43± 1.22	20.13± 0.62	24.03± 1.62
Petroleum ether extract (500mg/kg)	103.23 ±2.72	195.12 ±5.72	97.43± 2.52	25.23± 1.62	34.43± 1.32	31.53± 1.22	18.43± 1.22	18.13± 0.62	21.03± 1.62

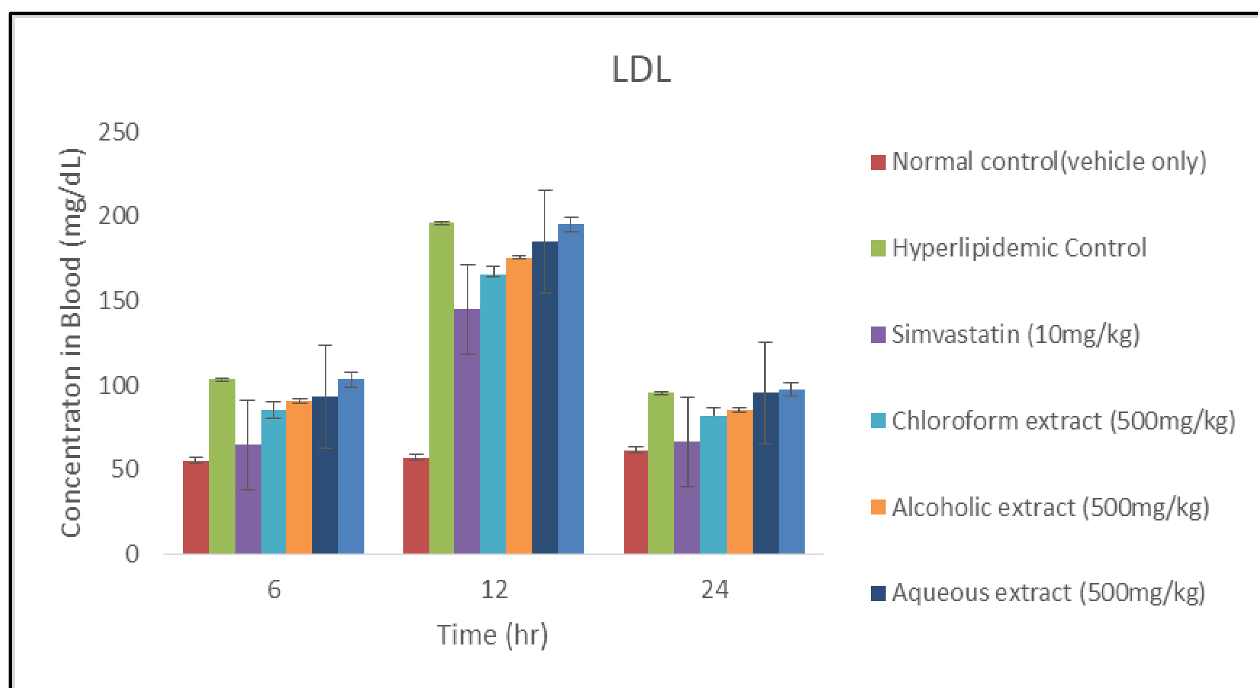

Figure 1: Effect of Chloroform, Alcohol and Aqueous extract of *E. ganitrus* leaves on LDL concentration in blood using Simvastatin induced hyperlipidaemia

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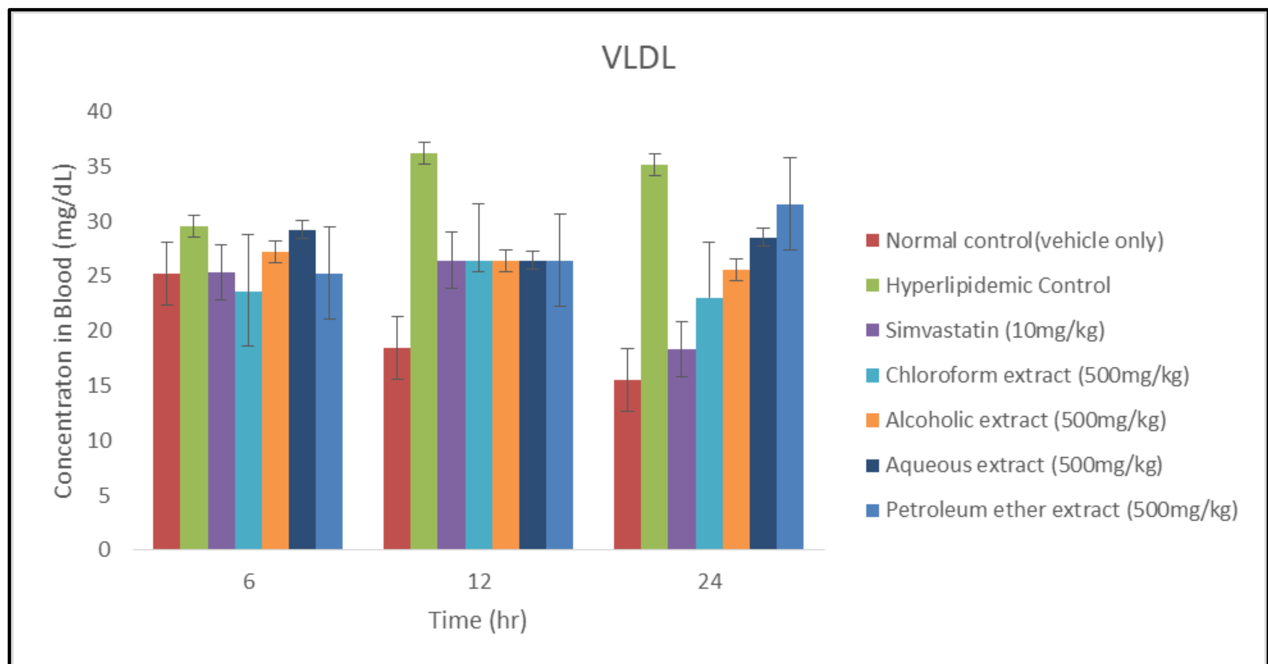

Figure 2: Effect of Chloroform, Alcohol and Aqueous extract of *E. ganitrus* leaves on VLDL concentration in blood using Simvastatin induced hyperlipidaemia

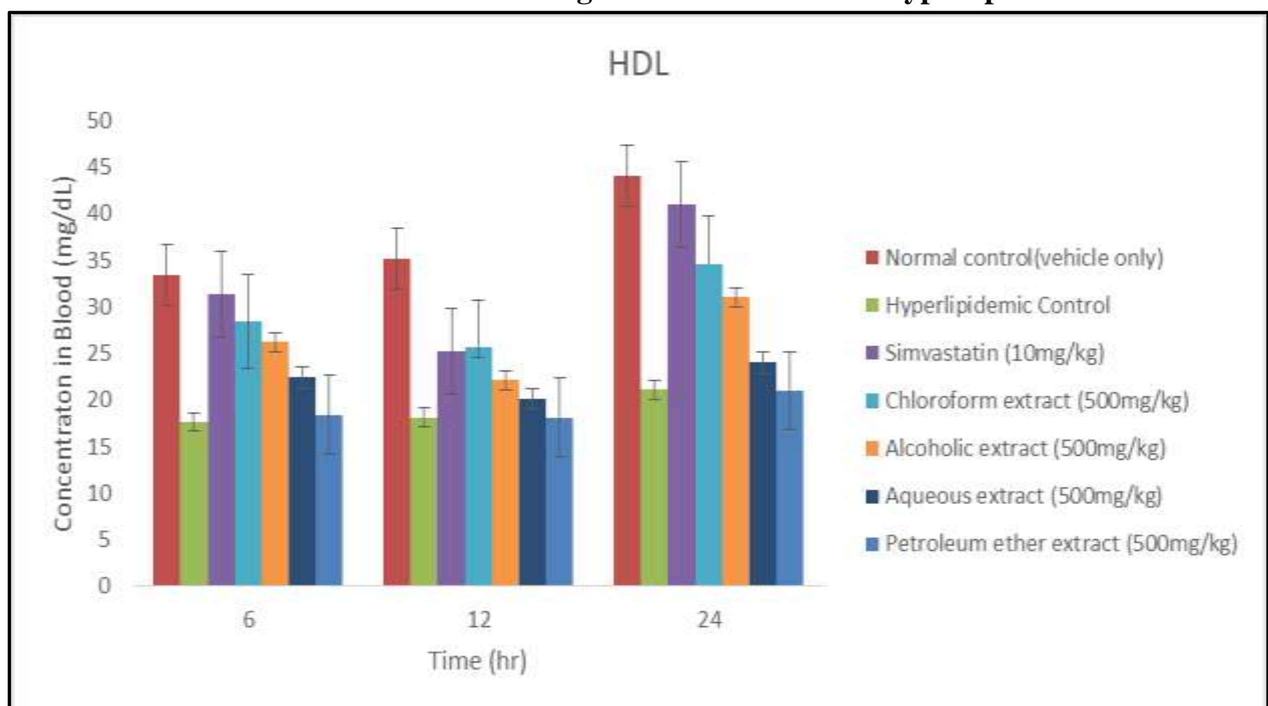

Figure 3: Effect of Chloroform, Alcohol and Aqueous extract of *E. ganitrus* leaves on HDL concentration in blood using Simvastatin induced hyperlipidaemia

CONCLUSION

Based on the findings, it was concluded that the selected medicinal plant extracts exhibited moderate to high antioxidant activity. Treatment with *E. ganitrus* leaves extract (500 mg/kg) substantially lowered cholesterol, triglycerides, phospholipids, VLDL and LDL levels

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compared to hyperlipidaemic control. These results suggest the leaves of *E. ganitrus* may be considered as a promising plant extract for the control of oxidation and hyperlipidaemia.

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