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### RESEARCH ARTICLE

#### ISOLATION OF PUNICIC ACID FROM POMEGRANATE SEED OIL, ITS CHARACTERIZATION AND EVALUATION FOR ANTIATHEROSCLEROSIS ACTIVITY.

\*Mr. Rahul Subhash Buchade<sup>1</sup>, Ms Aarati Ramesh Supekar<sup>2</sup>, Mr. Aniket Jaysingrao Kale<sup>3</sup>,  
Monika Gopal Shinde<sup>4</sup>

1.Department of Pharmachemistry, Alard College of Pharmacy, Pune.

2.Department of Pharmaceutical chemistry, Alard College of Pharmacy, Pune.

3.Sciformix Technology Ltd., Hinjewadi, Pune.

4.Research Scholar at BVU Poona College of Pharmacy, Pune.

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#### Abstract

**Purpose:-** To compare the diagnostic performance of digital breast tomosynthesis (DBT) and digital mammography (DM) for breast cancer. Materials and Methods: Ninety patients from December 2013 till December 2015 were enrolled in the study. All patients had pathologically proven cancer. Assessment of the lesions based upon digital tomosynthesis, compared with digital mammography (DM) and correlated with the pathological results. Results: BT shows better detection of malignant speculated masses (75% on DCI, 76.47% on ILC, 84.38% on IDC compared with (12.5%, 41.18%, 67.19% on DM respectively). Fourteen malignant masses were clearly visible on BT while were questionably visible on DM. BT changes BIRADS classification from IV to V in 22 patients (24.44% more accurate; 81.11/56.66%).

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#### Introduction:-

Lifestyle-related disease, such as obesity, hyperlipidemia, arteriosclerosis, diabetes mellitus & hypertension are widely spread & increasingly prevalent in industrialized countries. Because of rapid increase in the number of elderly people, these disease important medically & socio-economically. It is also assumed that one third of humans associated with this disease because of habits & lifestyle.

Literature search afforded several medicinal plant (single or polyherbal), extract and active constituents with significant anti – atherosclerosis activity; limited data is accumulated on such activity.

Epidemiologic studies have shown an inverse correlation between consumption of fish or other sources of dietary n-3 fatty acids & cardiovascular events. Dietary n-3 fatty acids prevent restenosis after percutaneous coronary angioplasty or induce regression of coronary atherosclerosis. Food source rich in n-3 fatty acids are thought to be beneficial in secondary prophylaxis after myocardial infarction. Pomegranate seed oil is one of the only plant sources of conjugated fatty acids & it contains abundant amount of fatty acid, a compound closely related to Conjugated Linoleic acid (CLA). The punicic acid found in pomegranate seed oil & has been called “Super CLA” whose effect is even more potent than ordinary CLA. Dietary supplementation of 1% CLA reduces the amount of

abdominal white adipose tissue, serum triglycerides level & liver TG level in animals. These effects were attributed to the enhanced fatty acid beta oxidation & the suppression of fatty acid synthesis in the liver.

### Material and Methods:-

**Corresponding Author:- Ms Aarati Ramesh Supekar.**

Address:- Department of Pharmacognosy, Alard College of Pharmacy, Pune.

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### Extraction of pomegranate seeds oil:-

Dried pomegranate seeds were pulverized & particles with size distribution of less than 40 mesh was used for the extraction. The extraction was carried out by using different extraction methods.

### Soxhlet Extraction:-

Two hundred grams of the crushed seeds were refluxed using two different organic solvents (Benzene & hexane (600 ml) in a soxhlet apparatus. The solvents were then evaporated in a vacuum oven at 35 ° C.

### Normal Stirring Extraction:-

Five grams of sample blended with 20 ml solvent (Benzene & hexane) & mixed thoroughly using magnetic stirrer for 4 h. The solvents were then evaporated in a vacuum oven at 35 ° C.

### Sonication & Extraction procedure:-

Five grams ground pomegranate seeds was mixed with 20 ml solvent (Benzene & hexane). The sample solvent suspension was then ultrasonicated for 45 min using ultrasonic bath at ambient conditions. The solvents were then evaporated in a vacuum oven at 35 ° C.

### Microwave-assisted Extraction:-

Five grams of crushed pomegranate seeds was mixed with 20 ml solvent (Benzene & hexane). The suspension was then irradiated in a household microwave oven (full power =1,000 W) for 30 s at different power levels (200,400,600,800 W), & the suspension was cooled to ambient conditions .This cycle was continued for a total heating time of 10 min, when oil extraction was completed & more heating time did not affect the extraction yield . Oil separation was then carried out by applying the vacuum oven at 35 ° C.

### Isolation of Punicic acid By Column Chromatography:-

The parent pomegranate oil extracted by the various extraction processes from pomegranate seeds was then fractionated by using the solvents according to polarity in ascending order i.e. by using Hexane: Diethyl Ether. Then excessive solvent was evaporated by applying the vacuum oven at 35 ° C. The isolation & separation of punicic acid from pomegranate seeds oil was done by using the silica gel (mesh size 100-250) column with hexane: diethyl ether tap (90:10). Previously the slurry of silica gel was prepared with the mobile phase. The column was washed with the mobile phase for sufficient period of time. Then 2.0 gm of pomegranate oil fraction was loaded over the silica gel. The mobile phase was passed continuously with constant flow rate (10 ml / min.). The fraction were collected at regular intervals of time, Then excessive solvent was evaporated by applying the vacuum oven at 35 ° C.

### Characterisation of Punicic acid:-

#### Thin layer Chromatography:-

In TLC of punicic acid Silica Gel –G plate used as stationary phase, Hexane: Diethyl ether (94:6) used as mobile phase, detection were done by vanillin sulphuric acid reagent & scanning done by densitometer at 254 nm. Spot of Punicic acid visualized as blue, blue-violet color.

#### Ultra visible Spectroscopy (UV):-

UV were taken out in the cyclohexane solvent for the Punicic acid, UV spectrophotometer (Shimadzu 1601) From this we get the  $\lambda$  and scanning results. (Graph No.1)

#### Infrared Spectroscopy (IR):-

Infrared spectrophotometer is used for the analysis of the wavelength. Punicic acid isolated by using the column. An IR spectrum is taken out for that Punicic acid (Graph No. 2). From that we get specific group peak which gives idea about basically present in to the sample.

### **Atherosclerosis Activity:-**

#### **Triton X- 100 induced hyperlipidemia:-**

Adult ratus norvegicus rats of either sex weighing 150-200 gm are taken for the anti-hyperlipidemic activity. They are fed with standard pallet diet & given water ad libitum for 7 days. The animals are divided in six groups of six animals each.

The animals were administered test compound & standard drug for seven consecutive days via intragastric tube once daily. On 8<sup>th</sup> day the animals fasted for 18 hrs (had only access to water) & triton X- 100 dissolved in 0.9 % saline was injected intravenously. After 8<sup>th</sup> day of treatment (after 18 hrs injection of triton X- 100), the blood was collected by retro orbital sinus puncture, under mild ether anesthesia. Serum obtained by immediate centrifugation of blood samples using ultra cooling centrifuge at 3000 rpm for 15 min at room temperature. Plasma was quantified using enzymatic kit.

#### **Statistical analysis:-**

Values were presented as mean  $\pm$  S.E.M. Statistical analysis were performed by one way ANOVA followed by Dunnett's multiple comparison using INTA software.

### **Result & Discussion:-**

The extraction of Pomegranate seed oil were performed by different extraction methods such as soxhlet, Normal stirring, Sonication, Microwave assisted using benzene –hexane solvent mixture & Percentage yield was found to be 19.542 % w/w, 9.96 % w/w, 16.16 % w/w, 21.30 % w/w respectively.

#### **Column Chromatography:-**

The extracts obtained by different extraction method were used for isolation of fatty acids by using column chromatography (Table No. 1). The total yield & percentage yield of Punicic acid was found to be 1.9 mg & 0.4561 % respectively.

**Table 1:-** Total yield & % yield of isolated fatty acids.

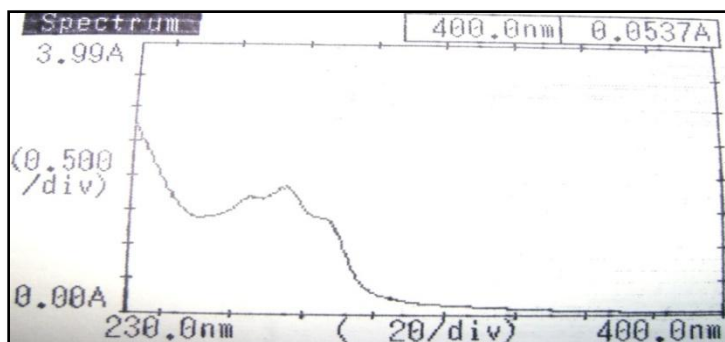
Sr. no	Isolated fatty acid	Total Yield	% Yield
1)	Punicic acid	1.9 mg	0.4561
2)	Dihydroxyteric acid	1.09 mg	0.91
3)	Linolic acid	0.94 mg	0.72
4)	$\beta$ - Elaeostaeric acid	0.67 mg	0.35
5)	Oleic acid	1.1 mg	0.23

#### **Characterization:-**

Thin Layer chromatography of Punicic acid was studied & Rf value was found to be 0.45 which indicate the presence of punicic acid.

#### **UV visible Spectroscopy (UV):-**

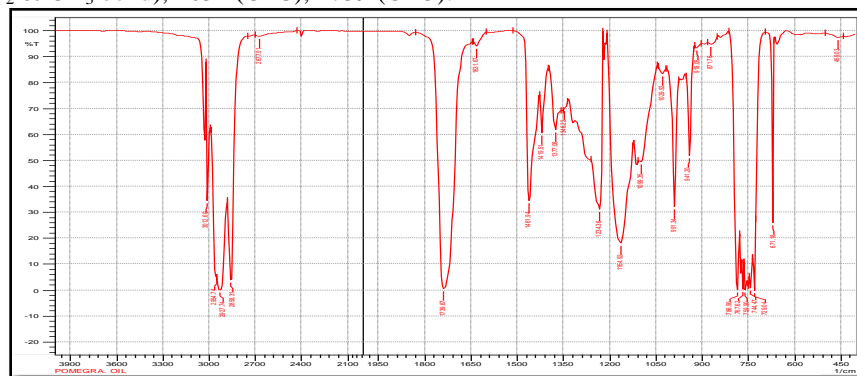
UV visible spectroscopy of isolated fraction were done at  $\lambda$  max 265nm, 275 nm, 287 nm, the absorbance were found 1.7112, 1.8600, 1.3651 respectively.



**Graph No. 1:-** The UV spectrum of Punicic acid isolated from pomegranate seed oil.

#### IR Spectroscopy:-

IR spectrum of isolated fraction was shows 918 (C-H bend), 941 (C-H bend), 991 (C-O stretch), 1346 (C-H bend in plane), 1461 (CH<sub>2</sub> & CH<sub>3</sub> bend), 1631 (C=C), 1739 (C=O).



**Graph 2:-** IR Spectrum of Punicic acid Isolated from pomegranate seed oil.

#### Triton X-100 induced hyperlipidemia:-

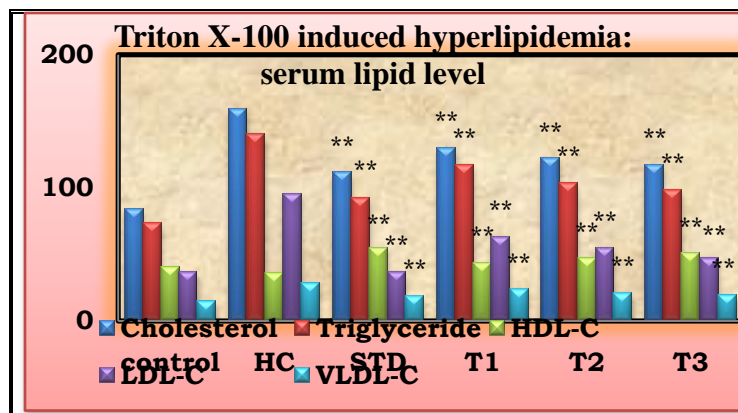
Triton X-100 has successfully induced hyperlipidemia in rats by increasing the serum TC, TG & LDL-C levels. Treatment with pomegranate seed oil at the doses of test 1, test 2, test 3 i.e. (250 mg, 500 mg, 750 mg / kg) reduced the serum TC, TG & LDL-C levels & increased the serum HDL-C levels when compared to the hyperlipidemic (HL) control group. It is widely accepted that reduction in plasma HDL-C levels is a risk factor for developing atherosclerosis. The change in lipid levels in groups of test 1, test 2, and test 3 were comparable with group of atorvastatin treated rats. Among three fractions, test 3 reduced the elevated lipid levels more significantly than the others.

**Table No. 2.0:-** Effect of pomegranate seed oil on serum lipid levels in triton induced hyperlipidemic rats.

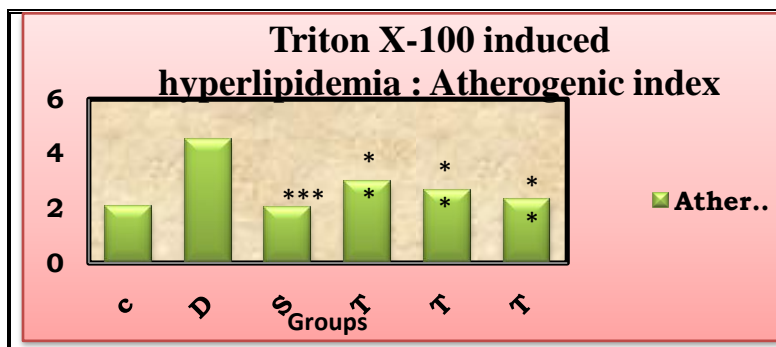
Groups	Control	HD	Standard	Test 1 250 mg/kg	Test 2 500 mg/kg	Test 3 750 mg/kg
Animals						
TC (mg/dl)	83.38± 0.047	158.90 ± 0.25	111.47 ± 0.35 **	129.62±_0.26 **	121.89 ± 0.30 **	117.08 ± 0.39 **
TG (mg/dl)	73.25± 0.029	140.03 0.10	92.13 ± 0.38 **	116.96± 0.54 **	103.24 ± 0.31 **	98.13± 0.28 **
HDL (mg/dl)	39.20± 0.11	35.33 ± 0.20	54.71± 0.30 **	43.39± 0.095 **	46.61± 1.68 **	50.66 ± 0.14 **
LDL (mg/dl)	29.53± 0.10	95.55 ± 0.22	36.99 ± 1.42 **	62.75 ± 0.31 **	54.62± 1.04 **	46.76± 0.33 **
VLDL(mg/dl)	14.65± 0.006	28.01 ± 0.019	18.43 ± 0.076 **	23.39± 0.076 **	20.64 ± 0.06 **	19.63 ± 0.056 **
A.I.	2.07	4.49	2.03 **	2.98 **	2.61 **	2.31 **

Where, N=6, Values are expressed as Mean ± SEM = Mean ± Std. error of mean.

\*p ≤ 0.05, \*\*p ≤ 0.01, \*\*\*p ≤ 0.001.



Graph No.3:- Effect of triton X-100 on blood serum lipid level of rat.



Graph No.4:- Atherogenic index of serum lipid level in triton X-100 induced hyperlipidemia

### Conclusion:-

The present study had shown an inverse correlation between consumption of CLA & cardiovascular events. Pomegranate seeds oil which was rich in Punicic acid significantly reduced oxidative stress by inhibiting the formation of oxidized LDL lipoproteins; macrophage lipid peroxidation & this mechanism atherogenesis were reduced. Anti-atherosclerosis activities were screened by the triton X-100 induced hyperlipidemic model in rats. Treatment with pomegranate seeds oil at a 750 mg/kg dose significantly reduced the serum TC, TG, & LDL-C levels & increased the serum HDL-C levels when compared to hyperlipidemic control. It was widely accepted that reduction in plasma HDL was a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism.

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