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ESTIMATION OF CHOLESTEROL, EGG CHOLESTEROL AND THE EFFECT OF ORANGE PEEL, GRAPE SEEDS AND GOOSEBERRY ON THEM USING RP-HPLC

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ABSTRACT

A simple, fast, specific and precise reverse phase high performance liquid chromatographic method (RP-HPLC) has been developed for the estimation of cholesterol, egg cholesterol and the effect of orange peel, grape seeds and gooseberry on them using RP-HPLC. Orange peel, goose berry and grape seeds are used to check whether they have any effect on reducing cholesterol by RP-HPLC method. Acetonitrile: 2 propanol (3:1) was selected as mobile phase. Detection wavelength of 215 nm was used to estimate cholesterol. Working standard solutions of concentrations 0.05-0.6 mg/ml were prepared for which a calibration graph was plotted having a correlation coefficient value of 0.999. This study may help to develop in the future a formulation that reduces cholesterol from these food materials.

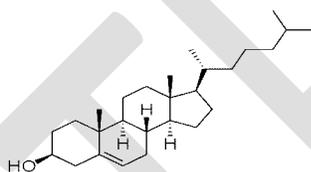
KEY WORDS: Cholesterol, Egg cholesterol, Goose berry, Grape seeds, Orange peel, RP-HPLC estimation.

INTRODUCTION:

Cholesterol (**Fig.1**) is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals¹. Cholesterol has a molecular formula of $C_{27}H_{46}O$. This molecule is composed of three regions (shown in the picture): a hydrocarbon tail, a ring structure region with 4 hydrocarbon rings, and a hydroxyl group. The hydroxyl (OH)

group is polar, which makes it soluble in water². Indian gooseberry seems to work by reducing total cholesterol levels, including the fatty acids called triglycerides, without affecting the s“good cholesterol” called high-density lipoprotein (HDL). In citrus peels that lower cholesterol levels it is a chemical like any other chemical.

Fig. 1: STRUCTURES OF CHOLESTEROL



The reason that citrus rind is said to have cholesterol-lowering effects is because it is known to contain PMFs which have been shown in various studies to offer numerous different benefits to individual health, including that of lowered cholesterol³⁻⁵. In studies of animals, certain dosages have been known to decrease the cholesterol levels of those with high cholesterol, bringing the levels back down to a more average range. However, studies on humans have been inconclusive, so it's not positive that polymethoxylated flavones

(PMFs) would have the same effect, long term, for people. Although the rinds of fruits like oranges do contain PMFs, they may not contain them in such a quantity as to make a difference in cholesterol levels⁶. A compound found in the peels of citrus fruit has the potential to lower cholesterol more effectively than some prescription drugs, and without side effects¹³. A compound found in the peels of citrus fruit has the potential to lower cholesterol more effectively than some prescription drugs.

MATERIALS AND METHOD:

All chemicals and reagents used were of analytical grade and were purchased from Merck Chemicals Corporation Ltd.

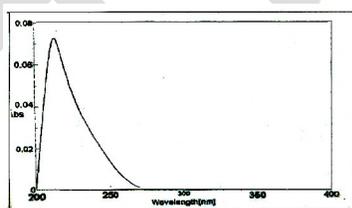
Mumbai, India. Deionized and ultra-pure water used in all experiments was obtained from Milli – Q system (Millipore).

SELECTION OF WAVELENGTH FOR ANALYSIS OF CHOLESTEROL:

The wavelength to be selected for the analysis of cholesterol was found out by UV Spectrophotometric method. Wavelength of 215 nm was selected for

the analysis of cholesterol, based upon the spectra obtained for pure cholesterol by UV Spectrophotometric method (Fig.2)

Fig.2: SPECTRAL ANALYSIS OF CHOLESTEROL



PREPARATION OF STANDARD STOCK SOLUTION:

50 mg cholesterol was accurately weighed into a 50ml standard flask and made up to the required volume with the mobile phase

acetonitrile: 2-propanol (3:1).the concentrations of this solution were mg/ml.

RECORDING OF A STANDARD CHROMATOGRAM:

After a study baseline was obtained, 20 μ l of the working standard solution was

injected into the chromatographic system, and the chromatograms were recorded.

Chromatographic conditions

Stationary Phase	: Waters Spherisorb ODS Column (4.6×150mm, 0.5 μ)
Pump	: LC-10 AT
Detector	: SPD 10 A
Elution method	: Isocratic
Flow rate	: 0.8 ml/min
Injection Volume	: 20μl
Detection Wavelength	: 215 nm
Mobile Phase	: Acetonitrile : 2-Propanol (3:1 v/v)
Retention time	: 11 min. (± 0.2)

EFFECT OF ORANGE PEEL, GRAPE SEEDS AND GOOSE BERRY ON STANDARD CHOLESTEROL:

Sample Collection and Preparation of solution:

Orange, grapes and gooseberry were obtained from shops in a local area. The orange peel and grape seeds were then separated. These were dried and powdered to fine. 10 mg of the powder were

accurately weighed and transferred into 10ml standard flasks respectively. The volume of which was made up to 10ml with acetonitrile: 2-propanol (3:1).

EFFECT ON CHOLESTEROL:

From the stock solutions of orange, grape and gooseberry 1ml was added into standard flasks containing the cholesterol of concentration 100, 300, 500 μg/ml. It

was mixed thoroughly and kept aside for 30 minutes. This solution was then analyzed for its cholesterol content at 30 minutes, 6 hrs and 24 hr intervals.

The chromatograms obtained for these food materials and the effect of orange peel, grape seeds and gooseberry on cholesterol are shown in **fig.3-5**.

Fig.3: Effect of Goose Berry on Standard Cholesterol

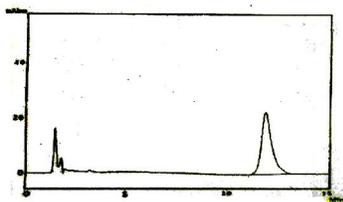


Fig.4: Effect of Grape Seeds on Standard Cholesterol

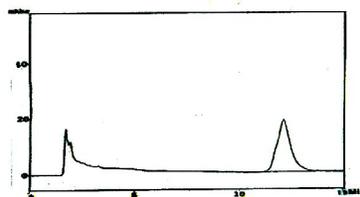
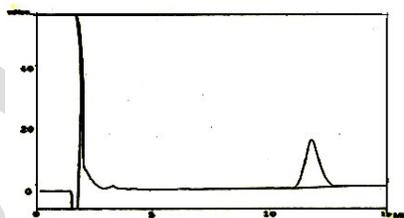


Fig.5: Effect of Orange Peel on Standard Cholesterol



PREPARATION OF EGG CHOLESTEROL SOLUTION:

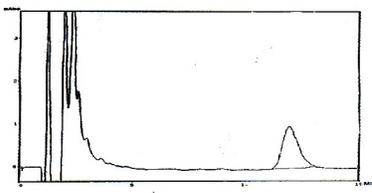
10 mg of yellow crystals obtained
was accurately weighed and transferred

into a test tube. 3 ml of hexane was then
added and mixed thoroughly.

The hexane solution was then decanted into another tube, into which nitrogen gas was passed at 35°C to evaporate the hexane. To the residue obtained 10 ml of mobile phase (acetonitrile: 2-propanol (3:1)) (solution B). This solution B was

then analyzed by RP-HPLC method using Acetonitrile: 2-propanol (3:1) as the mobile phase. The chromatogram (300 µg/ml) obtained for the extracted egg cholesterol are shown in **fig.6**

Fig. 6: Chromatogram Showing Extract Egg Cholesterol



EFFECT OF ORANGE PEEL, GRAPE SEEDS AND GOOSE BERRY ON EGG CHOLESTEROL USING RP-HPLC:

Orange peel, grape seeds, goose berry were obtained and solutions prepared as mentioned under standard cholesterol. 1ml each of orange peel solution was transferred into a 10 ml standard flask into which 1 and 5ml of the egg cholesterol (solution A) was added. The volume of the standard flasks was then made up with

mobile phase. These solutions were then mixed thoroughly and kept aside for 30 min. These solutions are then analyzed for their cholesterol content at 30 minutes, 6 hr and 24 hr intervals. The chromatograms (300 µg/ml) showing the effect of gooseberry, grape seeds and orange peel on egg cholesterol are shown in **fig 7-9**.

Fig.7: Effect of Gooseberry on Egg Cholesterol

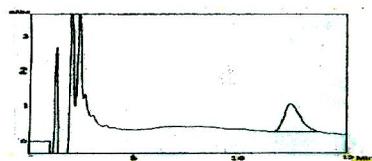


Fig.8: Effect of Grape seeds on Egg Cholesterol

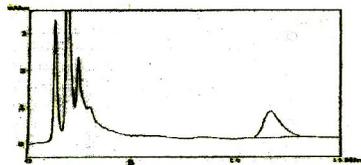
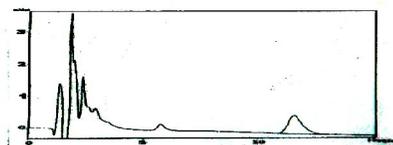


Fig.9: Effect of Orange Peel on Egg Cholesterol



RESULTS AND DISCUSSION:

CALIBRATION GRAPH:

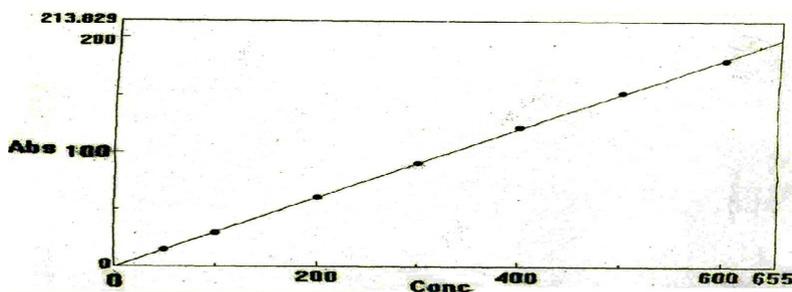
The calibration graph of standard cholesterol was plotted using peak area Vs concentration as shown in **fig. 10**. The

slope, intercepts and coefficient values were obtained to be 0.332, 0.308 and 0.9999 respectively (Table 1)

TABLE- 1: PEAK AREA OBTAINED FOR PURE CHOLESTEROL

Concentration(mg/ml)	Peak Area
0.05	1,50,686
0.10	2,93,916
0.20	6,03,382
0.30	9,05,409
0.40	12,24,080
0.50	15,23,761
0.60	18,07,019

Fig.10 : CALIBRATION GRAPH FOR STANDARD CHOLESTEROL



EFFECT OF FOOD MATERIALS ON STANDARD CHOLESTEROL:

The effects of orange peel, grape seed and gooseberry on standard cholesterol as a percentage reduction are summarized below. From the **Table 2**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 $\mu\text{g/ml}$ and time interval increases from $\frac{1}{2}$ -24 hrs. Therefore the highest percentage reduction

was obtained at a concentration of 100 $\mu\text{g/ml}$ after a time period of 24 hrs. On comparing the percentage reduction of 100-500 $\mu\text{g/ml}$ at 24 hrs its noticed that 100 $\mu\text{g/ml}$ showed 1.232 and 1.927 times more reduction than 300 and 500 $\mu\text{g/ml}$ respectively and 300 $\mu\text{g/ml}$ showed 1.56 times more reduction than 500 $\mu\text{g/ml}$.

TABLE – 2: EFFECT OF ORANGE PEEL ON CHOLESTEROL

Concentration ($\mu\text{g/ml}$)	Percentage reduction*		
	30 min	6 hrs	24 hrs
100	17.45	23.41	37.20
300	11.33	20.01	30.19
500	9.27	13.61	19.30

From the **Table 3**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 $\mu\text{g/ml}$ and time interval increases from $\frac{1}{2}$ -24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 100 $\mu\text{g/ml}$ after a time period of 24 hrs. On

comparing the percentage reduction of 100-500 $\mu\text{g/ml}$ at 24 hrs its noticed that 100 $\mu\text{g/ml}$ showed 1.691 and 2.93 times more reduction than 300 and 500 $\mu\text{g/ml}$ respectively and 300 $\mu\text{g/ml}$ showed 1.73 times more reduction than 500 $\mu\text{g/ml}$.

TABLE – 3: EFFECT OF GOOSE BERRY ON CHOLESTEROL

Concentration (µg/ml)	Percentage reduction *		
	30 min	6 hrs	24 hrs
100	14.99	18.65	32.38
300	10.82	14.32	19.15
500	7.96	9.49	11.06

*Average of three values

From the **Table 4**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 µg/ml and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 100 µg/ml after a time period of 24 hrs. On

comparing the percentage reduction of 100-500 µg/ml .Showed 2.208 and 2.81 times more reduction than 300 and 500 µg/ml respectively and 300 µg/ml showed 1.275 times more reduction than 500 µg/ml.

TABLE – 4: EFFECT OF GRAPE SEEDS ON CHOLESTEROL

Concentration (µg/ml)	Percentage reduction *		
	30 min	6 hrs	24 hrs
100	11.67	16.26	24.80
300	8.76	9.80	11.23
500	4.65	6.09	8.81

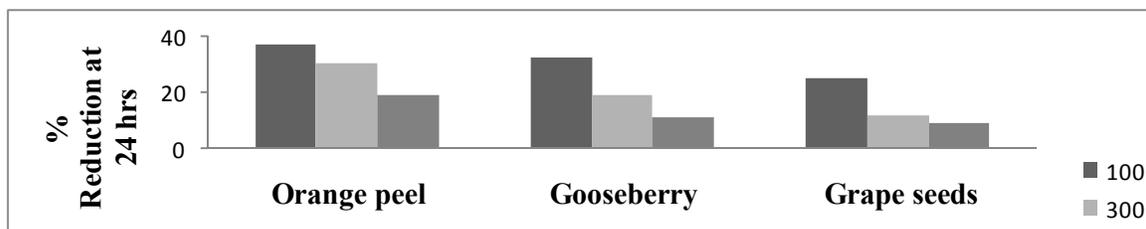
A comparison of the results of 100 µg/ml shows that orange peel gives 1.149 and 1.5 times more percentage reduction than

goose berry and grape seeds respectively and goose berry gives 1.306 times more percentage reduction than grape seeds.

Therefore it can be concluded that from among the three natural cholesterol fighters, orange peel was found to be most

effective followed by goose berry and then grape seeds (**fig.11**).

Fig.11: Percentage Reduction on Standard Cholesterol



EFFECT OF FOOD MATERIALS ON EGG CHOLESTEROL:

The cholesterol content estimated in egg yolk weighing 19 g was found to be 114mg/yolk. The effect of orange peel,

gooseberry and grape seeds on egg cholesterol as a percentage reduction are summarized below.

TABLE-5: EFFECT OF ORANGE PEEL ON EGG-CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
100	25.62	32.15	53.34
500	16.02	20.00	28.40

From the **Table 5**, it is observed that the percentage reduction it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 µg/ml and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 100 µg/ml after a time period of 24 hrs. On comparing the percentage reduction of 100-500 µg/ml at 24 hrs, it is noticed that 100µg/ml showed 1.87 times more

reduction than 500 µg/ml. From the **Table 6**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 µg/ml and time interval increases from ½-24 hrs.

Therefore the highest percentage reduction was obtained at a concentration of 100 µg/ml after a time period of 24 hrs. On comparing the percentage reduction of 100 -500 µg/ml at 24 hrs it's noticed that 100

100 µg/ml showed 2.237 times more reduction than 500 µg/ml.

TABLE -6: EFFECT OF GOOSE BERRY ON EGG-CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
100	22.12	29.50	47.08
500	12.52	15.10	21.05

From the **Table 7**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 500-100 µg/ml and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 100 µg/ml after a time period of 24hrs. On comparing the percentage reduction of 100-500µg/ml at 24 hrs it's noticed that

100 µg/ml showed 2.162 times more reduction than 500 µg/ml.

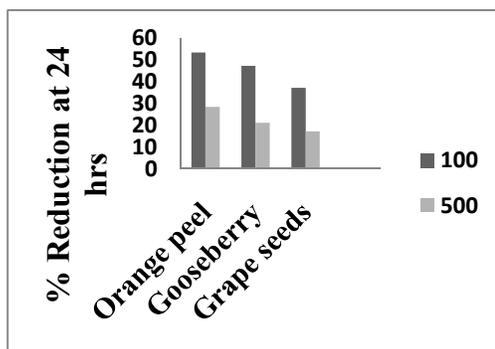
TABLE -7: EFFECT OF ORANGE PEEL ON EGG-CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
100	18.11	23.60	37.16
500	11.88	14.34	17.19

A Comparison of the results of 100 µg/ml shows that orange peel gives 1.133 and 1.435 times more percentage reduction than goose berry and grape seeds respectively and goose berry gives 1.267 times more percentage reduction than

grape seeds. **Therefore it can be concluded that from among the three natural cholesterol fighters, orange peel was found to be most effective followed by goose berry and then grape seeds. (fig.12)**

Fig. 12: PERCENTAGE RESUCTION ON EGG CHOLESTEROL



From the bar diagram **fig.13** it is evident it is evident that the percentage reduction for egg cholesterol is greater than that of standard cholesterol. Egg cholesterol

showed 1.434, 1.454 and 1.256 times more percentage reduction than standard cholesterol for orange peel, gooseberry and grape seeds respectively.

Fig. 13: COMPARISION OF THE PERCENTAGE REDUCTION AT 24 HRS FOR STANDARD AND EGG CHOLESTEROL



CONCLUSION:

All the three food materials showed a reduction of cholesterol at different time intervals on varying concentrations. But the highest percentage reduction given by all the food materials was noticed to be at 24 hrs period on the least concentration. Of all the food materials used for the study orange peel was found to be most

effective. It showed 1.149 times more percentage reduction than goose berry on cholesterol. It also gives 1.133 times more percentage reduction than goose berry on egg cholesterol. Orange peel was found to be 1.50 times more reduction than grape seeds on cholesterol.

It also gave 1.896 times more percentage reduction than grape seeds on egg cholesterol. Goose berry was found to give 1.306 times more percentage reduction than grape seeds on cholesterol. It also gave 1.267 times more percentage reduction than grape seeds on egg cholesterol. From the above summary it

can be concluded that orange peel reduced cholesterol and egg cholesterol more than goose berry and grape seeds. Therefore, orange peel can be used for further studies on cholesterol reduction and also it may be used in the development of a cholesterol reducing formulation.

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