ISSN: 2230-7346
Available online http://WWW.JGTPS.COM
Research Article Journal of Global Trends in Pharmaceutical Sciences
Vol.2, Issue 1, pp -63-79, January–March 2011

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

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ABSTRACT

A simple, rapid, precise and accurate HPTLC method was developed for the estimation of cholesterol. Acetonitrile: 2 propanol (3:1) was employed as the solvent. The wavelength for the estimation was selected as 215 nm. The mobile phase fixed after optimization was Toluene: Acetone: Glacial acetic acid (6:1.3:0.1 v/v/v). The R_f value was found to be 0.55 ± 0.03. This study may help to develop in the future a formulation that reduces cholesterol from these food materials.

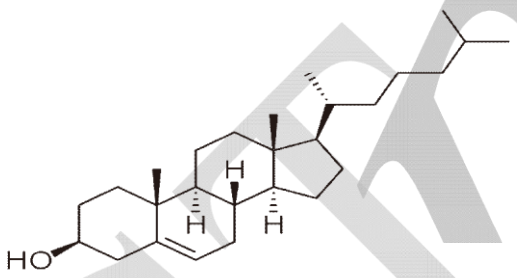
KEY WORDS: Cholesterol, Egg cholesterol, Goose berry, Grape seeds, Orange peel, HPTLC estimation.

INTRODUCTION:

Cholesterol (**Fig.1**) is a waxy steroid metabolite found in the cell membranes and transported in the blood plasma of all animals[1]. Cholesterol has a molecular formula of $C_{27}H_{45}OH$. 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 water[2].Indian gooseberry seems to work by reducing total cholesterol levels, including the fatty acids called triglycerides, without affecting the "good cholesterol" called high-density lipoprotein (HDL). In citrus peels that lower cholesterol levels it is a chemical like any other chemical. 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 cholesterol[3-5].

Fig. 1: STRUCTURE OF CHOLESTEROL



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 Poly Methoxylated 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 levels[6-12]. A compound found in the peels of citrus fruit has the potential to lower cholesterol more effectively than some prescription drugs, and without side effects[13-17]. A compound found in the peels of citrus fruit has the potential to lower cholesterol more effectively than some prescription drugs.

MATERIALS AND METHODS:

Chemicals:

All other 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).Silica gel 60F_{254} TLC plates (20×10 cm & 10×10 cm, layer thickness 0.2mm, Merck, Germany) were used as stationary phase.

Equipment:

The instrument used in the present study was Camag HPTLC system comprising Camag Linomat V automatic sample applicator, Hamilton syringe (100µl), Camag TLC scanner III with Wincats software. The HPTLC system consisted of Linomat V auto sprayer connected to a nitrogen cylinder, a twin trough glass chamber (10×10 cm), saturated with filter paper for ten minutes.

HPTLC METHOD AND CHROMATOGRAPHIC CONDITIONS:

Selection of solvent:

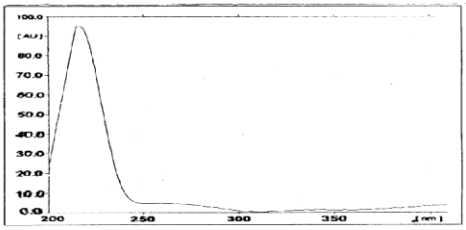
Cholesterol is freely soluble in acetone, chloroform, ether and ethyl acetate, sparingly soluble in alcohol and insoluble in water. Acetonitrile: 2- propanol (3:1 v/v) was selected.

Selection of wavelength:

The sensitivity of the HPTLC method that uses UV detection depends upon the proper selection of wavelength. An ideal wavelength is one that gives maximum absorbance and good response for the drug to be detected. UV spectrum of Cholesterol on TLC pre-coated plate showed maximum

absorption at 215 nm which was selected as the detection wavelength (**Fig. 2**)

Fig.2 UV SPECTRUM OF CHOLESTEROL



Preparation of Standard cholesterol Solution:

Standard stock solution was prepared by dissolving 62.5 mg of cholesterol in acetonitrile : 2-propanol (3:1) in a 25 ml standard flask and the volume was made up to 25 ml with acetonitrile : 2-propanol (3:1) to obtain a concentration of 2.5 mg/ml. From this 2,4,6,and 8 ml were taken in 10 ml standard flask and volume made up to 10 ml with the same to obtain a concentration of 0.5, 1.0, 1.5, 2.0 µg/ml.

Preparation of egg cholesterol solution:

Cholesterol is isolated from egg. 25 mg of this cholesterol was weighed and 10 ml of hexane was added. Then it was mixed thoroughly and the hexane layer was decanted into another test tube. Nitrogen gas was then passed through the tube at 35 degree Celsius to evaporate all the hexane. To the residue obtained 10 ml of acetonitrile : 2-propanol (3:1) was added, to obtain a stock solution of concentration 2.5mg/ml. 8ml of the stock was taken in a 10 ml standard flask and the volume was made up with acetonitrile : 2-propanol (3:1) making the concentration 2.0 µg/ml.

Recording the chromatogram:

With the fixed chromatographic conditions all the standard cholesterol solutions of concentration 0.5 to 2.5 µg/ml were applied on the plate, dried, developed, analyzed photometrically and chromatograms were recorded. The R_f value of the standard was found to be 0.55 ± 0.03. Calibration curves were plotted using peak areas of standard

cholesterol v/s concentration of the standard solution. This is followed by the application of egg cholesterol solutions of 2.0 to 25 µg/ml . The peak areas of the sample chromatograms were compared and the amount of cholesterol in egg was calculated.

Prewashing of plates:

HPTLC was performed on 10×10cm pre-coated silica gel $60F_{254}$ pre-coated plates from E-Merck. The adsorbent has a very large surface area; it may absorb air and other impurities from atmosphere, particularly volatile impurities, after the pack has been opened. The non-volatile impurities adsorbed by layer can lead to irregular baseline in scanning densitometry. To avoid possible interference from such impurities in quantitative analysis, plates were prewashed with methanol, dried, and activated for 30 minutes at 110 degree centigrade with the plates being placed between two sheets of glass to prevent deformation of the aluminum during heating.

Sample application:

The samples of Cholesterol were spotted on pre-coated TLC plates in the form of narrow bands of lengths 6mm, with 10mm from the bottom and left margin and with 9 mm distance between two bands. Samples were applied under continuous drying stream of nitrogen gas at constant application rate of 150 nLs^{-1}.

Mobile phase and migration:

Various solvent systems like mixture of

a) Chloroform: formic acid: butyl acetate

b) Chloroform: methanol: ammonia

c)Ethyl acetate: methanol: ammonia: formic acid e) Butyl acetate: acetic acid: hexane

f) Toluene: ethyl acetate were tried to separate and resolve spot of Cholesterol from its impurities and other excipients of formulation. The mixture of toluene: acetone: glacial acetic acid (6:1.3:0.1 v/v/v) could resolve with better peak shape. The drug was satisfactorily resolved with R_f value 0.55±0.03. Pre-saturation of TLC chamber with mobile phase was carried out for 30 minutes.

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

1ml of orange peel solution was transferred into working standard cholesterol solutions of concentration 1.5 – 2.5µg/ml and into the 2.0 and 2.5 µg/ml solution of egg cholesterol. These solutions were then mixed thoroughly and kept aside for 30 min. The same procedure was repeated for grape seed and gooseberry solutions. These solutions were then spotted and developed as mentioned above at 30 minutes, 6 hr and 24 hr intervals. The chromatograms showing the effect of orange peel, grape seeds and gooseberry on cholesterol both in standard and egg are shown in **fig.4-10**.

Fig. 4: STANDARD CHOLESTEROL WITH ORANGE PEEL

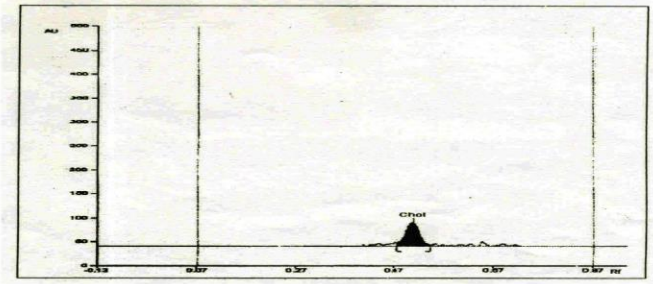


Fig.5: STANDARD CHOLESTEROL WITH GRAPE SEEDS

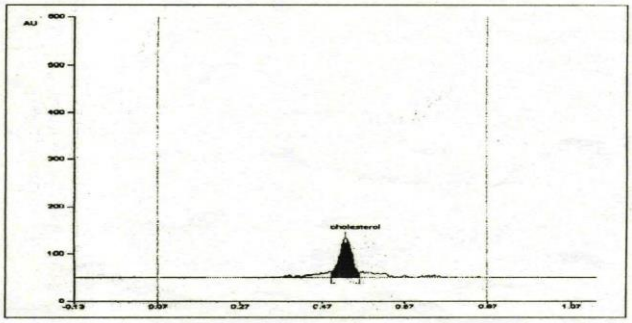


Fig.6: STANDARD CHOLESTEROL WITH GOOSEBERRY

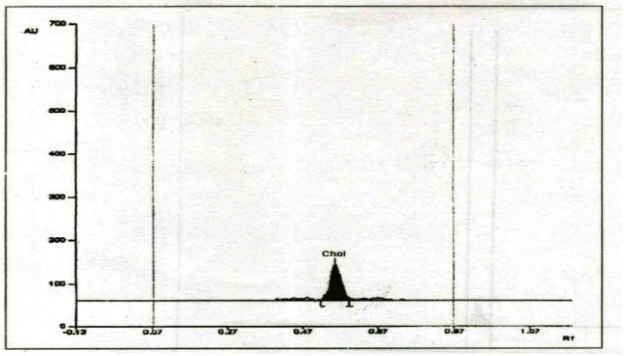


Fig.7: EGG CHOLESTEROL WITH ORANGE PEEL

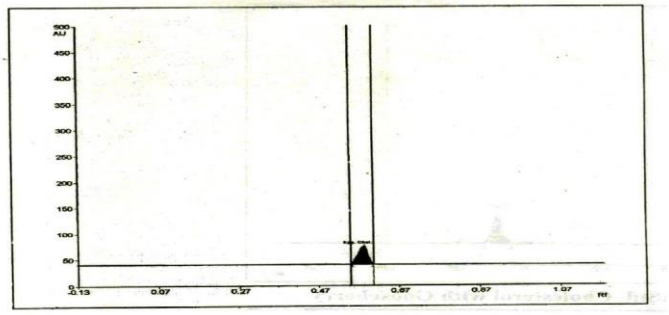
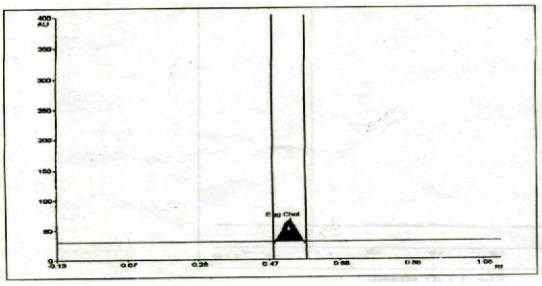


Fig.8: EGG CHOLESTEROL WITH GRAPE SEEDS



RESULTS AND DISCUSSION:

The effects of orange peel, grape seed and gooseberry on standard cholesterol as a percentage reduction are summarized below.

EFFECT OF FOOD MATERIALS ON STANDARD CHOLESTEROL:

TABLE: 1 EFFECT OF ORANGE PEEL ON CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
15	16.35	19.10	31.00
20	11.40	15.91	22.46
25	8.54	11.25	18.71

***Average of three values**

From the **table 1**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-15 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 15 µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 25-15 µg/ml at 24 hrs it is noticed that 15 µg/ml showed 1.380 and 1.657 times more reduction than 20 and 25µg/spot respectively and 20 µg/spot showed 1.200 times more reduction than 25 µg/spot.

TABLE: 2 EFFECT OF GOOSE BERRY ON CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
15	13.06	16.21	27.56
20	9.14	11.40	20.61
25	6.35	8.19	15.06

***Average of three values**

From the **table 2**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-15 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 15 µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 25-15 µg/spot at 24 hrs it is noticed that 15 µg/spot showed

1.337 and 1.830 times more reduction than 20 and 25µg/spot respectively and 20 µg/spot showed 1.369 times more reduction than 25 µg/spot.

TABLE: 3 EFFECT OF GRAPE SEEDS ON CHOLESTEROL

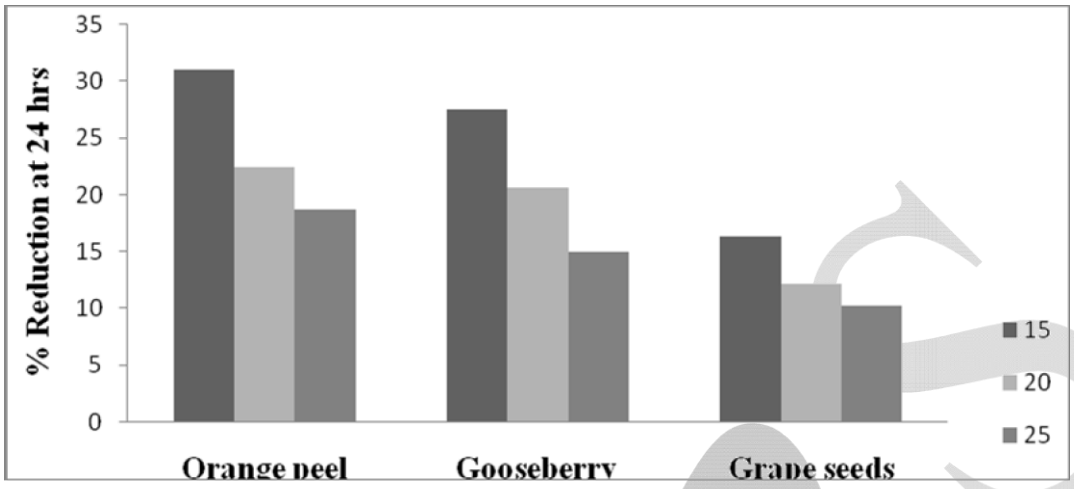
Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
15	9.71	12.86	16.35
20	7.82	9.47	12.18
25	4.14	6.59	10.28

***Average of three values**

From the **Table 3**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-15 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 15 µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 25-15 µg/spot at 24 hrs it is noticed that 15 µg/spot showed 1.342 and 1.590 times more reduction than 20 and 25µg/spot respectively and 20 µg/spot showed 1.185 times more reduction than 25 µg/spot.

A comparison of the results of 15 µg/spot shows that orange peel gives 1.125 and 1.896 times more percentage reduction than gooseberry and grape seeds respectively and gooseberry gives 1.686 times more reduction than grape seeds. The percentage reduction on standard cholesterol at 24 hrs was shown in **fig.11.** Therefore it can be concluded that from **among the three food materials, orange peel was found to be most effective followed by gooseberry and then grape seeds.**

Fig.11: PERCENTAGE REDUCTION ON STANDARD CHOLESTEROL



EFFECT OF FOOD MATERIALS ON EGG CHOLESTEROL

The cholesterol content estimated in egg yolk weighing 18 gms was found to be 110 mg/yolk. The effect of orange peel, gooseberry and grape seed on egg cholesterol as a percentage reduction are summarized below. From the **table 4**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-20 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 20 µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 20-25 µg/spot at 24 hrs it is noticed that 20 µg/spot showed 1.440 times more reduction than 25µg/spot.

TABLE : 4 EFFECT OF ORANGE PEEL ON EGG-CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
20	23.00	35.49	52.20
25	19.15	25.04	36.24

***Average of three values**

From the **table 5**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-20 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 20 µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 20-25 µg/spot at 24 hrs it is noticed that 20 µg/spot showed 1.633 times more reduction than 25 µg/spot.

TABLE: 5 EFFECT OF GOOSE BERRY ON EGG-CHOLESTEROL

Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
20	22.13	31.07	44.90
25	14.91	19.64	27.50

***Average of three values**

From the **table 6**, it is observed that the percentage reduction goes on increasing as the concentration of the cholesterol decreases from 25-20 µg/spot and time interval increases from ½-24 hrs. Therefore the highest percentage reduction was obtained at a concentration of 20µg/spot after a time period of 24 hrs. On comparing the percentage reduction of 20-25 µg/spot at 24 hrs it is noticed that 20 µg/spot showed 1.649 times more reduction than 25 µg/spot.

TABLE : 6 EFFECT OF GRAPE SEEDS ON EGG-CHOLESTEROL

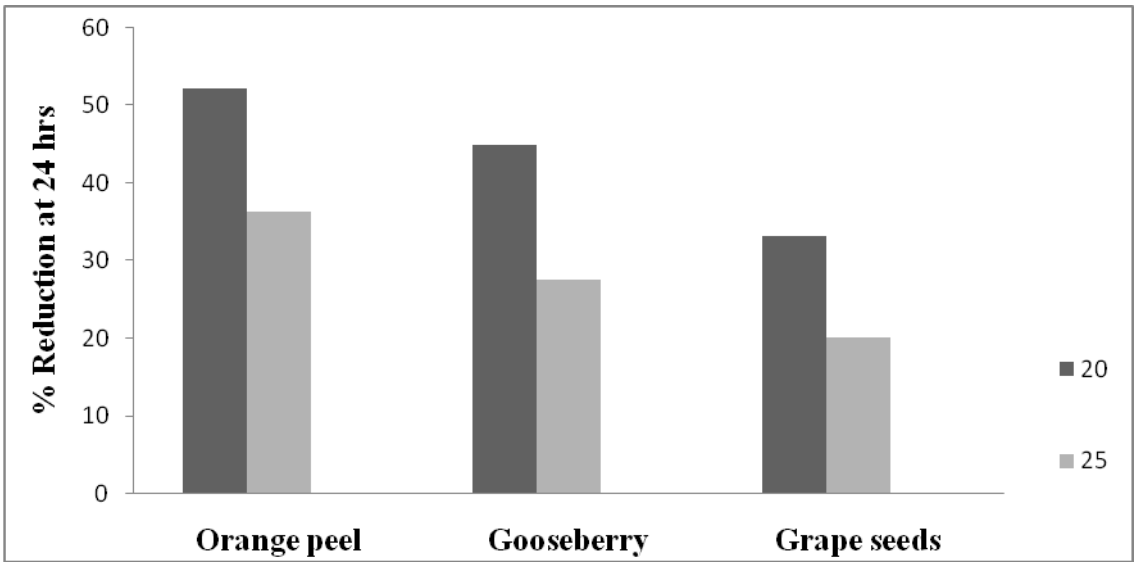
Concentration (µg/ml)	Percentage reduction*		
	30 min	6 hrs	24 hrs
20	17.51	20.2	33.14
25	10.90	13.66	20.10

***Average of three values**

A comparison of the result of the results of 20 µg/ml shows that orange peel was 1.074 and 1.575 times more percentage reduction than goose berry and grape seeds

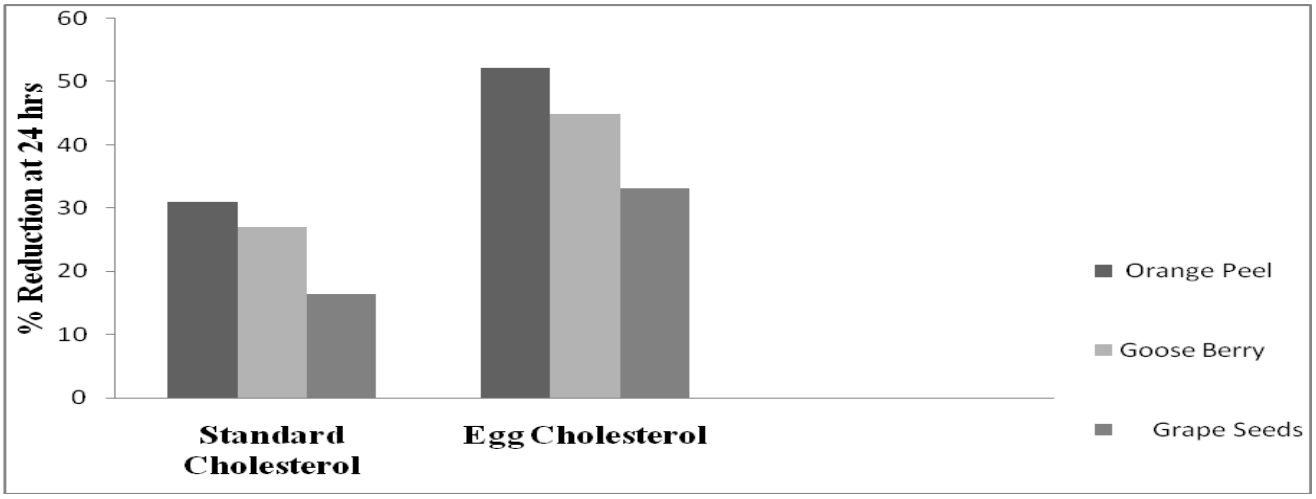
respectively and goose berry gives 1.355 times more reduction than grape seeds. The percentage reduction on egg cholesterol at 24 hrs was shown in **fig.12.** Therefore it can be concluded that from **among the three natural cholesterol fighters, orange peel was found to be most effective followed by goose berry and then grape seeds.**

Fig.12: PERCENTAGE REDUCTION ON EGG CHOLESTEROL



From the bar diagram **fig.13** it is evident that the percentage reduction for cholesterol is greater than that of standard cholesterol. Egg cholesterol showed 1.684, 1.629 and 2.027 times more percentage reduction than standard cholesterol for orange peel, goose berry and grape seeds respectively.

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



CONCLUSION:

All the food materials used in the study, orange peel was found to be most effective. It showed 1.149 and1.125 Times more percentage reduction than gooseberry on cholesterol in HPLC and HPTLC method respectively. It also gave 1.133and 1.074 times more percentage reduction than goose berry on egg cholesterol in HPLC and HPTLC respectively. Orange peel was found to give 1.50 and 1.435 times more percentage reduction than grape seed on cholesterol in HPLC and HPTLC method respectively. It also gave 1.896 and 1.575 times more percentage reduction than grape seed on egg cholesterol in HPLC and HPTLC methods respectively. Goose berry was found to give 1.306 and 1.686 times more percentage reduction than grape seeds on cholesterol in HPLC and HPTLC method

respectively. It also gave 1.267 and 1.355 times more percentage reduction than grape seeds on egg cholesterol in HPLC and HPTLC methods respectively. 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.

The percentage reduction both in HPLC and HPTLC were found to be more or less similar, so both the chromatographic techniques can be used for the determination of cholesterol and effect of other food materials on cholesterol. A comparison of the percentage reduction obtained in HPLC and HPTLC was shown in **table 7**.

TABLE: 7 comparison of the percentage reduction obtained in HPLC and HPTLC

Food Materials	Percentage reduction*			
	HPLC		HPTLC	
	Standard Cholesterol	Egg Cholesterol	Standard Cholesterol	Egg Cholesterol
Orange peel	37.26	53.34	31.00	52.00
Goose berry	32.38	47.08	27.56	44.90
Grape seeds	24.80	37.16	16.35	33.14

***Obtained at 24 hrs on the least concentration**

REFERENCES:

1. Emma Leah (May 2009). "Cholesterol" Lipidomics Gateway.

2. "Phylogenetic and biochemical evidence for sterol synthesis in the bacterium Gemmata obscuriglobus". Proc. Natl. Acad. Sci. U.S.A. 100 (26): 15352–7.

3. P.D. Seth, Qualitative analysis of drugs and formulations, 4th edition, 1996, Pp. No. 1-19.

4. Bielory L. Complementary and alternative interventions in asthma, allergy, and immunology. *AnnAllergy Asthma Immunol*. 2004; 93(2 Suppl 1):S45-54.

5. Busserolles J, Gueux E, Balasinska B, et al. In vivo antioxidant activity of procyanidin-rich extracts from grape seed and pine (Pinus maritima) bark in rats. *Int J Vitam Nutr Res.* 2006;76(1):22-7.

6. Chan MM, Mattiacci JA, Hwang HS, et al. Synergy between ethanol and grape polyphenols, quercetin, and resveratrol, in the inhibition of the inducible nitric oxide synthase pathway. Biochem Pharmacol. 2000; 60(10):1539-1548.

7. Chou EJ, Keevil JG, Aeschlimann S, et al. Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. Am J Cardiol. 2001;88(5):553-555.

8. Décordé K, Teissèdre PL, Sutra T, Ventura E, Cristol JP, Rouanet JM. Chardonnay grape seed procyanidin extract supplementation prevents high-fat diet-induced obesity in hamsters by improving adipokine imbalance and oxidative stress markers. *Mol Nutr Food Res*. 2008 Nov 26. [Epub ahead of print]

9. Fitzpatrick DF, Bing B, Maggi DA, et al. Vasodilating procyanidins derived from grape seeds. *Ann N Y Acad Sci.* 2002; 957:78-89.

10. Freedman JE, Parker C 3rd, Li L, et al. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. Circulation. 2001; 103(23):2792-2798.

11. Gruenwalkd J, Brendler T, Jaenicke C. PDR for Herbal Medicines, 4th ed. Montvale, NJ: Thomson Healthcare; 2007:405-410.

12. Hung LM, Chen JK, Huang SS, et al. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. Cardiovasc Res. 2000; 47(3):549-555.

13. Hu H, Qin YM. Grape seed proanthocyanidin extract induced mitochondria-associated apoptosis in human acute myeloid leukemia 14.3D10 cells. *Chin Med J* (Engl). 2006; 119(5):417-21.

14. Joshi SS, Kuszynski CA, Bagchi D. The cellular and molecular basis of health benefits of grape seed proanthocyanidin extract. Curr Pharm Biotechnol. 2001; 2(2):187-200.

15. Bagchi D, Sen CK, Ray SD, et al. Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract. *Mutat Res.* 2003; 523-524:87-97.

16. Kaur M, Agarwal R, Agarwal C. Grape seed extract induces anoikis and caspase-mediated apoptosis in human prostate carcinoma LNCaP cells: possible role of ataxia telangiectasia mutated-p53 activation. *Mol Cancer Ther*. 2006;5(5):1265-74.

17. Kaur M, Mandair R, Agarwal R, Agarwal C. Grape seed extract induces cell cycle arrest and apoptosis in human colon carcinoma cells. *Nutr Cancer*. 2008; 60 Suppl 1:2-11.