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EXPERIMENTAL STUDY ON LIPID LOWERING EFFECT OF COMMONLY USED SPICES

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ABSTRACT

Aim of the study: The study determine the Antihyperlipidemic activity of *Cuminum cyminum(sveta jiraka), Trigonella foenum(methika), Foeniculum vulgare* (*misreya*)and its phytochemical constituents contributing antihyperlipidemia. **Materials and Methods:** The individual plant extract and bio-active formulation (2:1:1) were subjected to HPTLC finger print analysis which provided chemical evidences for the lipid lowering effect of selected drugs by revealing the presence of Luteolin & Quercetin that are proven lipid lowering flavones. **Results and Conclusions:** The data of the results depicted that the herbal formulation in the ratio of 2:1:1 possess antihyperlipidemic potential. Further in depth studies could result in the development of a safe and efficacious ayurvedic antiobese formulation.

KEYWORDS: *Cuminum cyminum, Trigonella foenum, Foeniculum* vulgare, High fat diet, Luteolin.

INTRODUCTION

Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases and cerebral vascular diseases. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease.[1] Hyperlipidemia is characterized

by elevated serum total cholesterol, low density lipid protein; very low density lipid protein and decreased high density lipoprotein levels. Obesity is a chronic disorder of carbohydrate and lipid metabolism and is increased by an increase fat deposition in adipose tissue and other internal organs. Obesity leads to the development of insulin resistance which in turn leads to type II diabetes, coronary heart diseases.[2] Medicinal plants play a major role in hypolipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestine.[3] In many developing countries, most hyperlipidemic individuals use medicinal plants as folk medicine to treat hyperlipidemia and prevent atherosclerosis. Therefore, there is a strong interest, locally, in natural hypolipidemic substances derived from medicinal plants.

Cumin (*Cuminum cyminum*) is an aromatic plant belongs to the Apiaceae family and is used to flavor foods, impart fragrances and is used in medicinal preparations. Cumin seeds contain 14.55% lipids. The seeds also reported to contain fourteen flavonoid glycosides of which seven belongs to apigenin, five to luteolin and two to chrysoeriol groups.[4] Essential oil present in cumin seeds has antihydrolytic effect and is better than conventional antioxidants like BHT. Cumin seeds decrease significantly the plasma levels of cholesterol and triglycerides. *C.cyminum* supplementation has a significant reduction in plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides.[5]

Fenugreek (*Trigonella foenum graecum L*.) is an annual herb belongs to family Fabaceae is widely grown in India. The seed contains four flavonoid and two steroidal saponins. An alkaloid trigonelline is present in the seed in a concentration of 0.38%. reports suggest hypolipedimic ctivity of trigonelline in high fat fed rats. Alkaloids, saponins and flavonoids of fenugreek seeds act as antilipidemic, hypoglycemic and hypercholesterolemia because chemical evidence shows promising results in reducing serum cholesterol levels.[6]

Fennel (*Foeniculum vulgare Mill*), a perennial herb belongs to Apiaceae family is cultivated worldwide. The percentage of oil varies considerably being lowest in fruits of Indian origin (0.7-1.2) and highest from Eastern Europe (4-6%) also contains fixed oils (9-13%). Trigonelline and choline were also present. F. vulgare has been reported to contain phenolic acids like 3-O-Caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 1,3-O-di-caffeoylquinic acid, 1,4-O-di-caffeoylquinic acid, 1,5-O-di-caffeoylquinic acid. The flavonoids like eriodictyol-7-rutinoside, quercetin-3-rutinoside and rosmarinic acid have also been isolated from F. vulgare.[7] Flavonoids and polyphenols may also contribute to the

hypolipidimic activity by increasing the cholesterol metabolism and by modulating the enzymes involved in cholesterol metabolism such as HMG COA reductase, lecithin cholesterolacyl transferase, cholesterol7-alpha hydroxylase and acyl COA.[8]

MATERIALS AND METHODS

The present study was undertaken to investigate the antihyperlipidemic effect of a polyherbal formulation using *Cuminum cyminum, Trigonella foenum, Foeniculum vulgare* using HPTLC.

Collection of plant materials

The seeds of Cumin, Fennel and Fenugreek were purchased from Local market, Thanjavur and its identity was authenticated by department of CARISM, SASTRA University, Thanjavur.

Chemicals

Quercetin, Luteolin was procured from Sigma Aldrich and Natural Remedies. All other chemical are of AR grade.

Extract preparation

The Fruits and seeds of cumin, fenugreek, fennel were pulverized to a coarse powder and macerated with 30% ethanol and kept it for 3 days with frequent shaking. After complete extraction, the samples were filtered and evaporated over a water bath at low temperature. The dried extract was used for standardization, formulation and analytical studies.

Quantitative analysis

Some active metabolites like phenols, flavonoids, alkaloids and tannins in hydro-alcoholic extract of cumin, fennel, fenugreek and its combination were estimated by different chemical methods.

Hptlc analysis

The qualitative HPTLC analysis was performed with standard solution of luteolin, quercetin were compared with individual extracts and its formulation. The diluted samples and standards were spotted on silica gel $60F_{254}$ (10x10cm) of 0.2mm thickness (E.merck, Dermstadt, Germany) using Linomat5 (CAMAG, Switzerland) automatic sample applicator. The composition of the mobile phase used for the identification of luteolin and quercetin were toluene: ethyl acetate: formic acid (10:9:1) and toluene: ethyl Acetate: methanol: formic

acid (6:3:0.2:0.4). After development, the plates were dried in hot air oven at 105°C for 5 min and scanned using TLC Scanner3 (CAMAG, Switzerland). The chromatogram was visualized and photographed under UV light at λ=254 and 366nm using CAMAG Reprostar3 (CAMAG, Switzerland) with winCATS software version of 1.3.4.

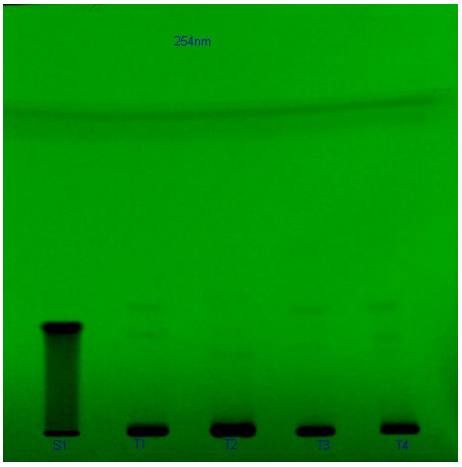
Statistical analysis

Statistical comparisons were performed using GraphPad Prism 6.07 (Trial version) by one-way analysis of variance (ANOVA), followed by post hoc Tukey's test. Results were expressed as mean ± S.E.M. and $p < 0.05$ was considered significant.

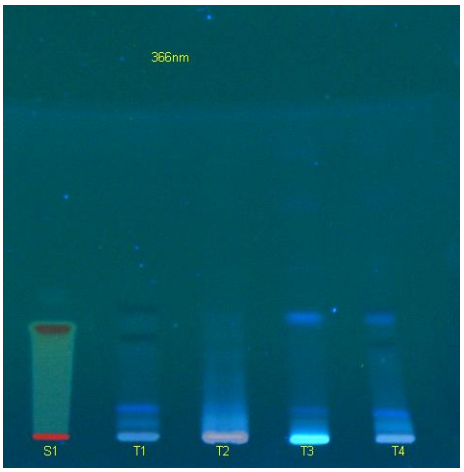
RESULTS

Table 1: Quantitative analysis of individual extracts and its active formulation.

S.No.	Extract	Total phenols (%)	Total Flavonoids (%)	Total Alkaloids (%)	Total Tannins (%)
1.	*Cuminum cyminum*	3.044	1.9243	0.9145	0.6512
2.	*Foeniculum vulgare*	3.393	2.6208	1.477	0.4975
3.	*Trigonella foenum graecum*	3.193	2.218	2.033	0.8098
5.	F2(2:1:1)	4.221	2.122	0.5179	0.9926

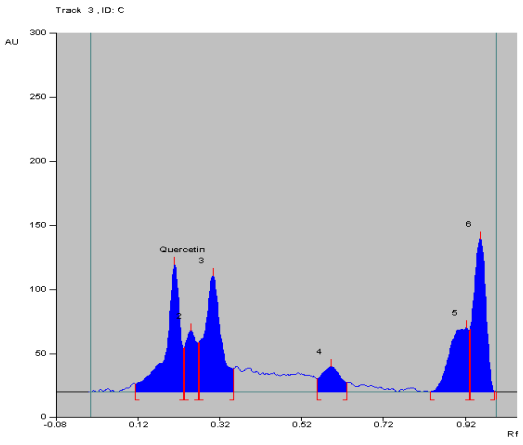


AT 254nm



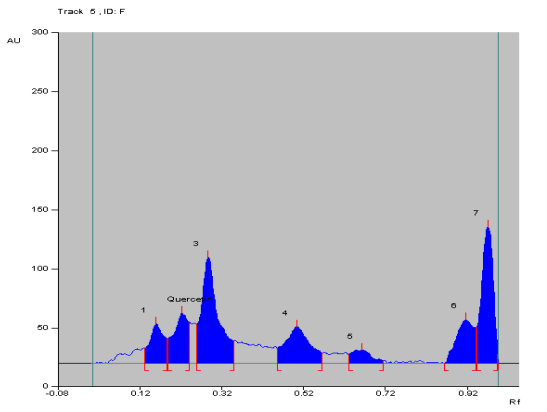
AT 366nm

PEAK DISPLAY (10µl of Sample-Cumin)



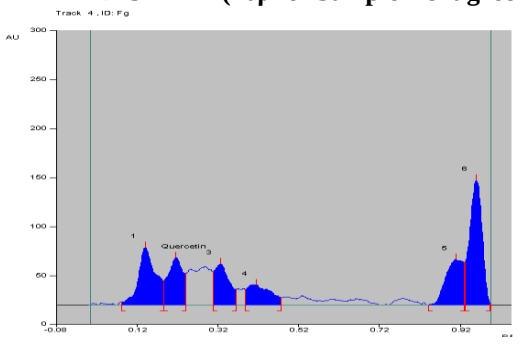
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	6.2	0.21	99.5	23.27	0.23	33.9	2452.5	22.73	Quercetin
2	0.23	34.6	0.25	47.6	11.14	0.27	38.4	923.7	8.56	unknown *
3	0.27	38.7	0.30	91.2	21.33	0.35	18.0	2560.2	23.72	unknown *
4	0.56	10.5	0.59	19.6	4.59	0.63	7.6	656.4	6.08	unknown *
5	0.84	0.0	0.92	50.1	11.72	0.93	47.9	1669.7	15.47	unknown *
6	0.93	48.6	0.96	119.5	27.95	0.99	0.8	2528.7	23.43	unknown *

PEAK DISPLAY (10µl of Sample-Fennel)



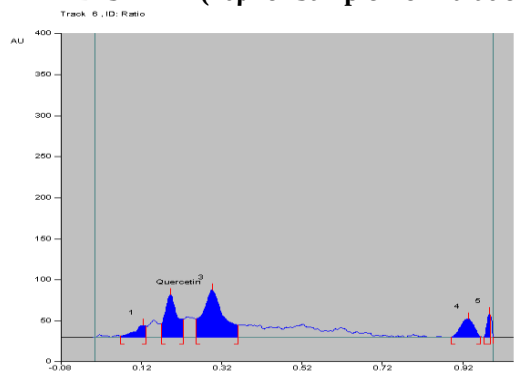
Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.13	12.9	0.16	33.4	9.25	0.19	21.0	824.6	8.51	unknown *
2	0.19	21.1	0.22	42.4	11.77	0.24	35.2	1062.0	10.95	Quercetin
3	0.26	33.1	0.29	89.9	24.93	0.35	19.0	2598.0	26.80	unknown *
4	0.46	12.8	0.50	30.9	8.57	0.57	8.8	1329.4	13.71	unknown *
5	0.63	7.6	0.66	11.4	3.16	0.71	2.5	405.0	4.18	unknown *
6	0.87	0.1	0.92	36.8	10.21	0.94	30.0	1133.5	11.69	unknown *
7	0.94	30.3	0.97	115.9	32.12	1.00	7.5	2343.0	24.17	unknown *

PEAK DISPLAY (10µl of Sample-Fenugreek)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	2.9	0.14	58.8	17.11	0.19	24.3	1785.1	19.29	unknown *
2	0.19	24.7	0.22	48.4	14.08	0.24	31.9	1261.3	13.63	Quercetin
3	0.31	34.0	0.33	41.8	12.16	0.36	15.7	1113.2	12.03	unknown *
4	0.39	16.0	0.41	20.7	6.03	0.48	7.4	865.8	9.35	unknown *
5	0.84	0.1	0.91	46.5	13.53	0.93	43.5	1426.3	15.41	unknown *
6	0.93	43.5	0.96	127.4	37.09	1.00	0.9	2804.4	30.30	unknown *

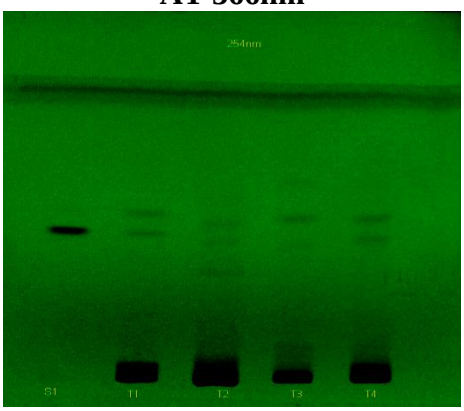
PEAK DISPLAY (10µl of Sample-Formulation)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.07	1.7	0.13	14.5	8.25	0.13	13.2	303.9	7.21	unknown *
2	0.17	16.2	0.19	52.3	29.73	0.22	21.8	1126.0	26.70	Quercetin
3	0.26	22.4	0.30	57.9	32.95	0.36	14.6	2070.4	49.10	unknown *
4	0.89	0.1	0.94	22.4	12.76	0.96	1.1	533.4	12.65	unknown *
5	0.97	0.2	0.99	28.7	16.31	0.99	26.9	183.3	4.35	unknown *

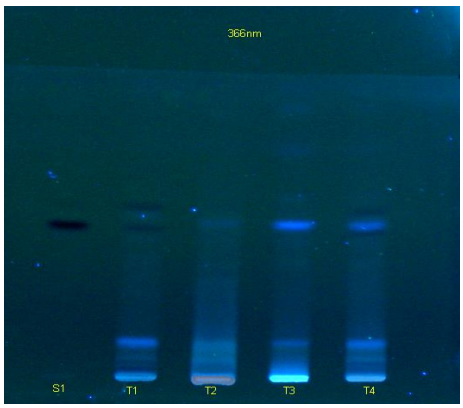
Fig. 1: HPTLC profile of individual extracts and its active formulation based on Quercetin.

AT 366nm

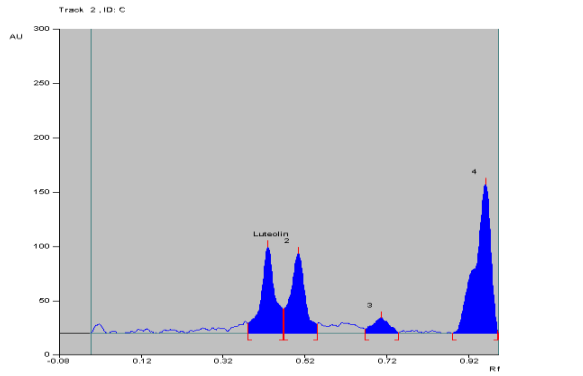


PEAK DISPLAY (10µl of cumin)

AT 254nm

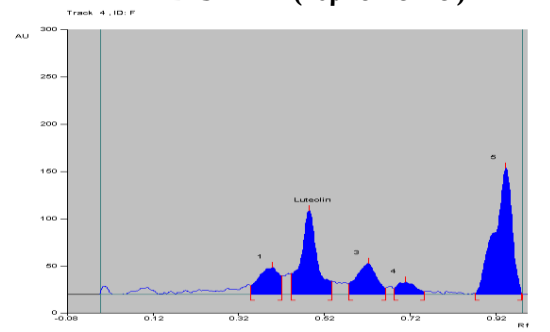


PEAK DISPLAY (10µl of fenugreek)

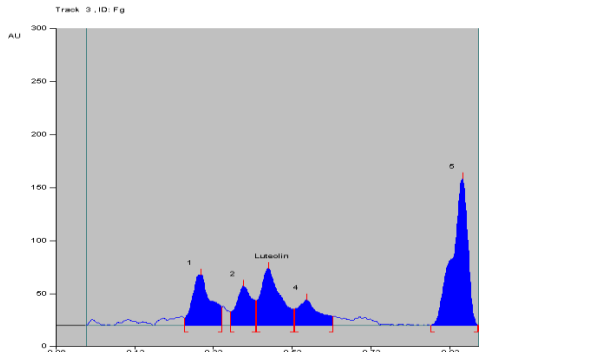


Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.39	9.3	0.44	79.7	28.45	0.47	22.6	1990.8	27.79	Luteolin
2	0.47	22.6	0.51	73.6	26.28	0.56	8.8	1912.1	26.69	unknown *
3	0.67	4.0	0.71	14.2	5.08	0.75	0.7	435.0	6.07	unknown *
4	0.90	0.2	0.97	112.6	40.19	0.99	8.8	2825.5	39.44	unknown *

PEAK DISPLAY (10µl of fennel)

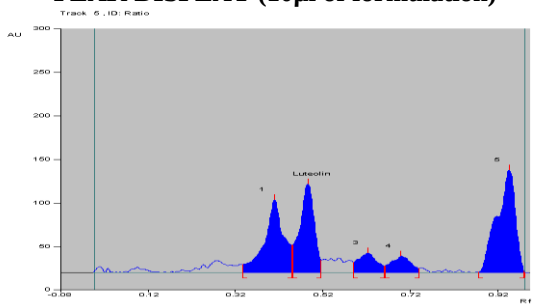


Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.35	9.4	0.40	28.6	9.62	0.42	19.5	1013.0	11.11	unknown *
2	0.45	22.0	0.49	88.6	29.86	0.54	13.9	2448.3	26.86	Luteolin
3	0.58	11.4	0.63	32.6	10.99	0.67	6.7	1149.1	12.61	unknown *
4	0.69	6.2	0.72	13.1	4.42	0.76	3.1	421.5	4.62	unknown *
5	0.88	1.2	0.95	133.9	45.11	0.99	1.5	4082.2	44.79	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.25	6.9	0.29	47.8	16.18	0.35	17.6	1695.9	17.75	unknown *
2	0.37	12.8	0.40	36.9	12.47	0.43	23.7	1107.6	11.59	unknown *
3	0.44	23.8	0.46	54.0	18.26	0.53	15.3	1984.2	20.77	Luteolin
4	0.53	15.4	0.56	24.2	8.18	0.63	8.4	981.5	10.27	unknown *
5	0.88	0.2	0.96	132.8	44.91	0.99	2.8	3784.1	39.61	unknown *

PEAK DISPLAY (10µl of formulation)



Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.34	9.5	0.42	84.3	24.32	0.46	31.6	2733.3	26.53	unknown *
2	0.46	31.8	0.49	102.3	29.51	0.52	14.4	2496.8	24.23	Luteolin
3	0.60	12.0	0.63	22.6	6.54	0.67	7.4	735.9	7.14	unknown *
4	0.67	7.4	0.70	19.0	5.48	0.74	4.7	635.6	6.17	unknown *
5	0.88	0.8	0.95	118.4	34.16	0.99	1.6	3701.8	35.93	unknown *

Fig. 2: HPTLC profile of individual extracts and its active formulation based on Luteolin.

DISCUSSION

Cardiovascular disease is a growing health concern in developing countries resulting in increasing the risk factors such as obesity, hypertension, diabetics and hyperlipidemia. The major risk factors involved in the development of cardiovascular disease are hypercholesterolemia and hypertriglyceridemia. Reducing the elevated cholesterol and triglycerides in plasma is the most effective methods in the treatment of atherosclerosis and its complications. There are many numbers of drugs available in market prescribed by physician to reduce the elevated cholesterol levels in hyper-lipidemic patients.[9] (Feng et al., 2011). The common adverse effects reported with fibrates includes GI disturbance, rash, headache, pancreatitis and myotoxicity and the drug is not recommended for renal and hepatic disorder patients. Fibrates in combination with statin derivatives, myopathies are

increased in several folds as compared with statin alone.[10,11] (Davidson et al., 2006, Mantri et al., 2014). Treatment of hyper-lipidemic patients with fibrates leads to elevation of LDL levels and this is due to the conversion of VLDL to LDL particles.[12] (Berglund et al., 2014). Antihyperlipedimic drugs such as fibrates and statins lose their demand in market because of its adverse effect and drug dependence property.[13] (Alsheikh et al., 2004). Now-a-days herbs and their formulation become much popular due to its minimal adverse effects and multiple targets in preventing and curing hyperlipidemia.

In the present study we have formulated four formulations containing different ratios of *Cuminum cyminum*[14] (Dhandapani et al., 2002) *Trigonella foenum*[15] (Mukthamba and Srinivasan, 2015) and *Foeniculum vulgare*[16] (Oulmouden et al., 2011).

In recent years, there has been increasing interest from western medicine in phytochemical combinations, which have been fundamental in traditional systems of herbal medicines. Combination of some molecules may synergistically increase their therapeutic activity. Interestingly in this context quercetin, luteolin with some other flavonoids, phenols and alkaloids can help to increase bioavailability. The identified luteolin and quercetin possesses catecholic B-ring, ortho hydroxyl group, the 3-hydroxy-4-keto group in quercetin and the presence of 5-hydroxy-4-keto group in both could significantly contribute the antioxidant and antihyperlipidemic activities of flavonoids.[17,18] (Rimbach et al., 2008, Odontuya et al., 2005).

CONCLUSION

For an effective and efficient antioxidant property for flavonoids, catecholic B-ring, hydroxyl groups in an ortho position, 3-hydroxy-4-keto and 5-hydroxy-4-keto is highly essential for scavenging free radicals. In this study, it was observed that total flavonoids content was found to be 2.12% in the herbal formulation (2:1:1) and flavanoids of luteolin and quercetin also identified by HPTLC from the bioactive formulation (2:1:1). HPTLC fingerprint analysis also proved the presence of quercetin and luteolin in the test substance. Further research work has to be done on pre-formulation studies to increase the stability and bioavailabilty of finished product.

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