CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1183297

Available online at: http://www.iajps.com Research Article

DETECTION AND QUANTIFICATION OF ANTI OXIDANT MARKERS IN MARKETED HEPATOPROTECTIVE FORMULATIONS BY HPTLC TECHNIQUE

Arivukkarasu R*, Rajasekaran A, Nadhiya R, Narmatha V.Nayanthara PS, Nithish K.
KMCH College of Pharmacy, Coimbatore, Tamilnadu, India-641048

Abstract:
The main objective is to detect and quantify the standard antioxidant markers in marketed hepatoprotective formulations by HPTLC method using finger print analysis. Results of the study clearly revealed that these six formulations contains flavonoids- quercetin, rutin and phenolic acid category- caffeic acid and gallic acid. The developed HPTLC method can be employed for the routine investigations of well-known free radical scavengers in marketed herbal hepatoprotective formulations.

Keywords: *Hepatoprotective, Anti oxidant, Caffeic acid, Quercetin, Rutin.*

Corresponding author:
Arivukkarasu R,
KMCH College of Pharmacy,
Coimbatore, Tamilnadu, India-641048
E-mail:phytoarivu@gmail.com,
Mobile +917094659909

QR code

Please cite this article in press as Arivukkarasu R et al., ***Detection and Quantification of Anti Oxidant Markers in Marketed Hepatoprotective Formulations by HPTLC Technique****, Indo Am. J. P. Sci, 2018; 05(02).*

INTRODUCTION:

The liver plays an important role in excretion bilirubin and metabolism of hormones, the detoxification of drugs and other toxins, and in the metabolic function so various nutrients including carbohydrates, proteins and fats [1]. However, hepatotoxic agents can induce almost all types of liver lesions by reacting with the basic cellular components. Infact, hepatocytes are exposed to orally administered xenobiotics via the portal venous circulation. Toxins and drugs are among the basic etiopathogenetic agents of acute liver failure in Western countries [2]. Besides, excessive consumptions of alcohol and viral infections are the most common risk factors for liver diseases in developed countries, while environmental pollution, hepatic viruses, parasitic infections and chemotherapeutics are the main factors known to cause hepatic damage in developing countries [3]. The 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane (p p -DDT),was the most used organo chlorine pesticide in the world. It was synthesized in 1874 and was discovered to be an effective insecticide in1939. It was first used mainly to protect military areas and personnel from vector-borne diseases such as malaria [4]. The use of DDT for malaria control was reduced or banned in the 1970s due to the emergence of environmental concerns such as its bio concentration and low degradability, replacements such as pyrethroid and bendiocarb were introduced. When DDT emissions ceased in 1990, about 634 kt DDT were released into the environment Stemmler et al., 2009 [23]. Even though the Stockholm Convention on Persistent Organic Pollutants listed DDT as the "Dirty Dozen" in 2001 for the global community UNEP 2017 [24]. DDT is still currently used in indoor residue spraying in 14 tropical countries and several other countries are preparing to reintroduce it van den Berg et al., 2009 [25]. Numerous analytical studies showed higher levels of DDT and its main metabolite DDE (dichlo-diphenyl ethylene) than the allowable daily intake in food [5] adipose tissues [6], maternal milk and lipid fractions of biological membranes all over the world. This fact is due to the high lipophility and bioaccumulation of DDTs [7]. Evidence accumulated over the years has suggested that chronic exposure to DDT and its derivatives is associated with loss weight, anorexia, sterility, endocrine disruptions, muscular weakness, tremors, hepatic effects, and anemia in humans [8]. Studies on liver toxicity, associated with acute DDT poisoning, include hepatomegaly, liver damage and liver function disorder [9-13].

DDT has many properties, it induces the synthesis of liver cytochrome P450 and related enzymes produces reactive intermediates during biotransformation [14] and elicits the development of liver tumors in rodents [15] and humans [16]. On other hand, it has been reported that oxidative stress which can result from either excess of reactive oxygen species (ROS) production and/or deficient antioxidant capacity, is involved in the toxicity of DDT in different biological systems like rat thymocytes[17] human hepatocytes [18] human blood mononuclear cells [19] gonads [20] rat lymphocytes, oral mucosa and breast cells [21]. Most of these studies have demonstrated the high efficacy of antioxidants like vitamin C and vitamin E, zinc, N-acetylcysteine in the protection of these different biological systems against p, p'-DDT cytotoxicity. Therefore, there is an urgent need for safe therapeutics options to treat DDT-induced liver pathology. Historically, plants have been used in the folk medicine to treat various diseases. Research showed that hepatoprotective effects have been associated with plant extracts that are rich in phenolic compounds because of their high antioxidant activities [22]. The most important traditionally practised plants used for hepatoprotective activity was *Tephrosia purpurea, Phyllanthus niruri, Eclipta alba, Andrographis paniculata, Picrorhiza kurroa, Solanum nigrum Piper longum, Terminalia chebula.* The plants above mentioned were scientifically proved for its action and phytocompounds responsible for therapeutic efficacy by various authors in reputed journals .Hence our aim to detect and quantify above said plants anti oxidant markers in various marketed herbal formulations by HPTLC Technique.

MATERIALS AND METHODS:

Collection of Marketed herbal formulations

Four formulations were procured from pharmacy shop, among them two were capsule dosage form, two were tablet dosage form namelyMF_1 (Liv First capsules) from Paalms health care,Madurai, ORF (we developed a formula funded by AICTE under RPS Scheme and patented, our Research Formulation – Livscore capsules) MF_2 Phyllanthus niruri from traditional practitioner, MF_3 (Hebeliv tablets) pondchy pharmaceuticals, Puduchery. MF_4 Ictrus capsules from Pharm products thanjavur.

Evaluation of Collected Marketed Herbal Formulations

Preparation of standards and extracts from the commercial herbal Formulations:

One gram of the each formulation was taken and sonicated with 10 ml of methanol. Filtered and the

filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml

Table 1: Ingredients in the marketed and our research formulations

S.No	**MF$_1$** (Livfirst capsules)	**MF$_2$** (Ttraditional formulations)	**MF$_3$** (Hebeliv tablets)	**MF$_4$** (Ictrus capsules)	**ORF** (our Research Formulation - Livscore capsules)
1.		*TephrosiaPurpurea*	*Tephrosia Purpurea*	-	-
2.	*Phyllanthus niruri*	*Phyllanthusniruri*	*Phyllanthus niruri*	*Phyllanthus niruri*	-
3.	*Eclipta alba*	*Eclipta alba*	*Eclipta alba*	-	-
4.	*Andrographis paniculata*	*Andrographispanic ulata*	*Andrographis paniculata*	-	-
5.	*Picrorhiza kurroa*	*Picrorhiza kurroa*	*Picrorhiza kurroa*	-	-
6.		*Solanum nigrum*	*Solanum nigrum*	-	-
7.		*Piper longum*	-	-	-
8.		*Terminalia chebula*	*Terminalia chebula*	-	-
9.		*Ocimum sanctum*		-	-
10.	-	-		*Ricinus communis*	-
11.	-	-		-	*Enicostemaaxill are*
12.	-	-	-	-	*Canscora heteroclita*

Table 2: Organoleptic evaluation and pH of formulations

Name of the formulation	**Colour**	**Odour**	**Nature of particles**	**Taste**	**P^H of 1% solution of formulation**
MF_1	Brown	aromatic	Fine powder	aromatic	6.2
MF_2	Brown	aromatic	Fine powder	aromatic	7.0
MF_3	Yellowish brown	aromatic	Fine powder	aromatic	7.3
MF_4	Brown	aromatic	Fine powder	aromatic	7.9
ORF	Brown	aromatic	Fine powder	Bitter with mucilagino us	6.9

Equipment:
A Camag HPTLC system comprising of Linomat 5 applicator and Camag TLC scanner and single pan balance of Shimadzu model was used, for weighing the samples.

Chemicals and solvents:
Rutin, Quercetin,gallic acid caffeicacid, ferulic acid and mangiferin were procured from Sigma Chemical Company Inc., USA. Solvents for extraction were purchased from Qualigens fine chemical (P) limited Mumbai. HPTLC was carried out using Merck aluminium sheet coated with silica gel GF 254 (0.2 mm).

Preparation of extracts from the commercial herbal Formulations:
One gram of the each formulation was taken and sonicated with 10 ml of methanol. Filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml

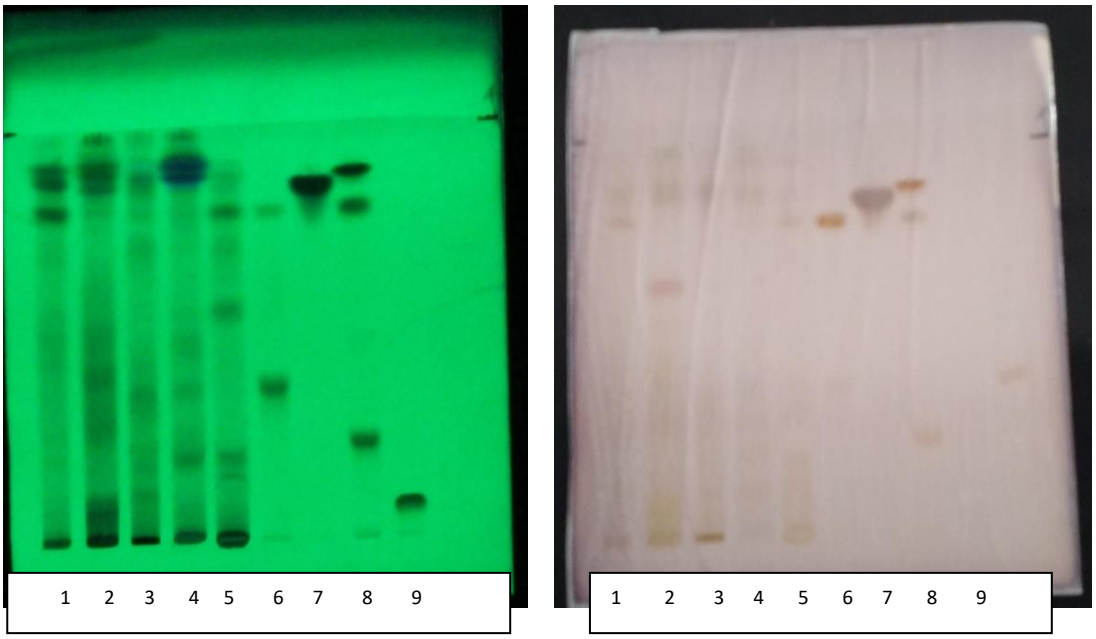
Application of sample:
The sample solutions were spotted in the form of bands of width 6 mm with a Hamilton 100 µl syringe on precoated plate 60 F254 (10 cm × 10 cm with 0.2 mm m thickness, E.Merck) using a CamagLinomat V applicator. The slit dimension was kept 6mm × 0.45 mm. 10 µl of each sample and standard solutions were applied on to the plate.

Development
The chromatogram was developed in Camag glass twin -through chamber (10× 10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid: methanol [3:6:1.6:0.4] for 10 min (temperature 25 ± 2°C, relative humidity 40%). The migration distance was 80 mm. TLC plates were air dried with air dryer. Densitometric scanning was performed using Camag TLC Scanner -III at 254 nm and 366 nm operated by a Wincat software.

Detection
The plate was scanned at UV 254 and 366 nm using CamagTLC Scanner-3 and Linomat V. $R_f$value of each compound which were separated on p late and data of peak area of each band was recorded.

RESULTS AND DISCUSSION:

The solvent composition tried for monitoring the elution of components in herbal formulations is Toluene: ethyl acetate: formic acid: methanol in the ratio of (3:6:1.6:0.4). The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometric analysis was performed at 254 nm in reflectance mode. The R_f values of the marker compounds were in the range of 0.05 to 0.82 (Table 3). MF_1showed Rf values same as that of Catechin 0.79, caffeic acid, 0.82, quercetin 0.85, ORF showed R_f values same as that of trigonilline0.06, Catechin 0.79, and 0.82.MF2 showed R_f value same as that of trigonilline 0.06,mangiferin 0.34. MF3 showed Rf value equal to trigonilline 0.06,gallic acid 0.77, and quercetin 0.85 MF_4 showed R_f value same as that of Catechin 0.77, quercetin 0.85. The chromatogram of the (MF_1, ORF MF_2, MF_3, and MF_4,) were shown in the Fig 2 to Fig 6. Fig 7 to Fig 10 shows standard markers. Thus we simultaneously detected and quantified the standard markers rutin, quercetin, gallic acid, mangiferin and catechin present in hepatoprotective commercial herbal formulations by HPTLC technique. Flavonoids and phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicines. The anti-oxidant activity of flavonoids is due to their ability to reduce free radical formation and to scavenge free radicals. Oxidative stress, the consequence of an imbalance of pro-oxidants and antioxidants in the organism is the key phenomenon in chronic illness like inflammatory diseases. Phytopharmaceuticals are gaining importance in modern medicine as well as traditional system of medicine owing to their therapeutic potential due to the presence of phytochemicals such as polyphenols, flavonoids and triterpenoids etc. Since they possess anti-inflammatiory, antioxidant, analgesic and cytostatic activity, the quantification of phytochemicals such as flavonoids and phenolics was necessary. Phenol and phenolic compound such as flavonoids have shown free-radical scavenging activity and protection against oxidative stress (Arivukkarasu et al., 2015). These secondary metabolites in plant possess potent antioxidant activity in terms of its radical scavenging activity. The antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers. Flavonoids ability of scavenging hydroxyl radicals and lipid peroxy radicals is important for prevention of diseases associated with oxidative damage of membranes, proteins and DNA. Hence in this effort we estimated the well-known free radical scavengers' rutin, quercetin and gallic acid in market herbal hepatoprotective formulations by HPTLC methods. This paper exposed that the levels of markers in formulations responsible for therapeutic activity.



1.MF1 (Livup) 2. ORF (LivScore) 3. MF2 (Phyllanthus trational formulation) 4. MF3 (Hebeliv) 5. MF4(Ictrus) 6.Catechin –Mangiferin 7.Caffeic Acid 8.Rutin & Quercetin & Gallic Acid 9.Trigonilline

Fig- 1: Chromatogram of four formulations and seven standards after development in mobile phase

Table 3: R_f values of free radical scavengers rutin, quercetin, gallic acid, Mangiferinand Caffeic acid in herbal formulations

Tracknumber/ Name of formulation	Amount of sample applied in µl	Number of peak	No.of compounds & its R_f values in formulations	Name of marker present in formulation
Track-1 MF_1	10.0 µl	12	0.07,0.10,0.20,0.26,0.31,0.36,0.43,0.49,0.67,**0.77**,**0.82**,0.85,0.89	Catechin **(0.79**)Caffeic acid**(0.82)** Quercetin **(0.85)**
Track-2 ORF	10.0 µl	8	0.07,0.28,0.39,0.49,0.61,**0.79**, **0.82**,**0.84**,0.87	Trigonilline**(0.06)**Catechin **(0.79)** Quercetin**(0.85)**
Track-3 MF_2 Phyllanthus	10.0 µl	11	**0.05**,0.10,0.17,0.21,**0.34**,0.40,0.54,0.62,0.70,**0.78**,**0.86**	Trigonilline **(0.06)** Mangiferin**(0.34**)
Track-4 MF_3	10.0 µl	10	**0.06**,0.19,0.35,0.46,0.53,0.62,0.69,**0.77**,**0.86**,0.88	Trigonilline(**0.06)** Gallic acid, **(0.77),** Quercetin**(0.85)**
Track-5 MF_4	10.0 µl	12	0.10,0.14,0.19,0.26,0.28,0.33,0.38,0.53,0.61,0.69,**0.77**,**0.85**	Catechin **(0.77)** Quercetin **(0.85)**
Track-6 Catechin–Mangiferin	10.0 µl	7	0.34, 0.77	Mangiferin(**0.34)** , Catechin **(0.77**)
Track-7 Caffeic Acid	10.0 µl	2	**0.82**	Caffeic acid (0.82)
Track-8 Rutin Gallic Acid, Quercetin-	15.0 µl	7	**0.20**,**0.77**,**0.85**	Rutin**(0.20)** Gallic acid (**0.77**)Quercetin**(0.85)**
Track-9 Trigonilline	10.0 µl	6	**0.06**.	Trigonilline**(0.06)**

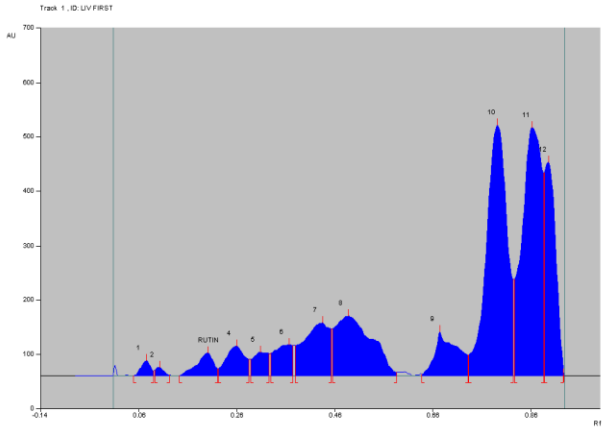


Fig-2: Chromatogram of MF_1

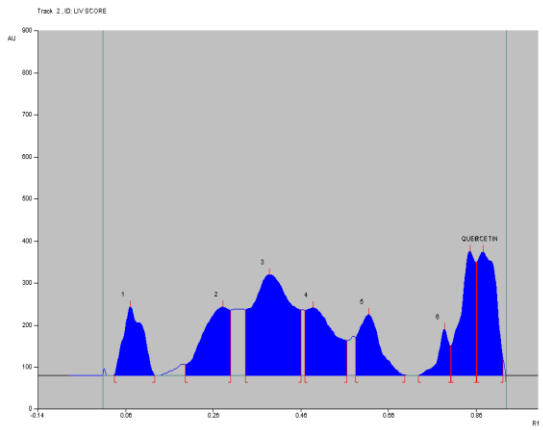


Fig- 3: Chromatogram of ORF

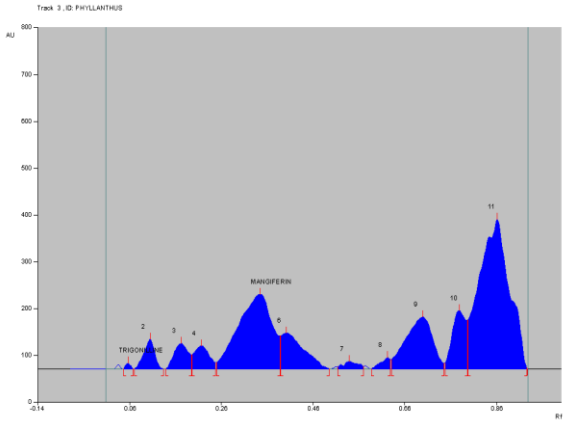


Fig- 4: Chromatogram of Phyllanthus

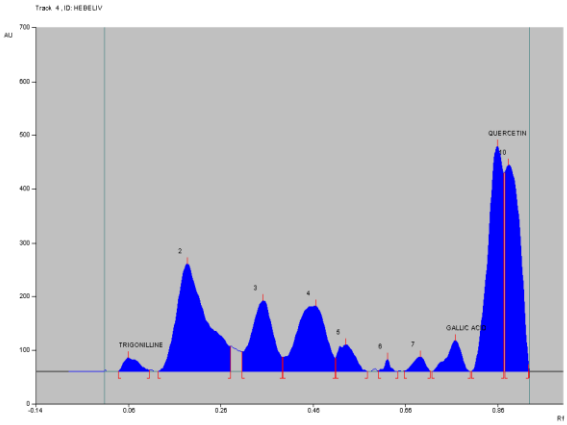


Fig- 5: Chromatogram of MF_4

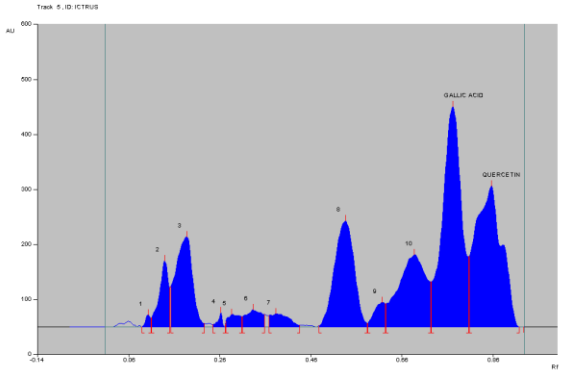


Fig- 6: Chromatogram of MF_5

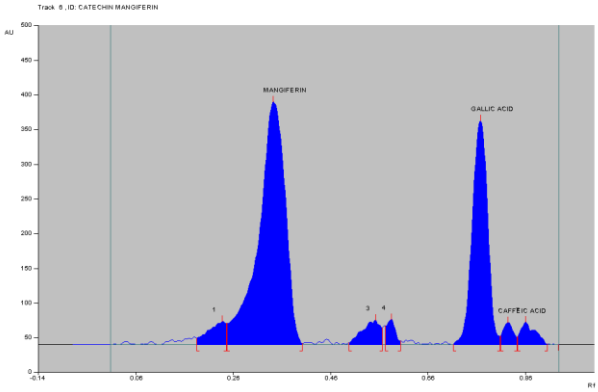


Fig- 7: Chromatogram of Catechin& Mangiferin

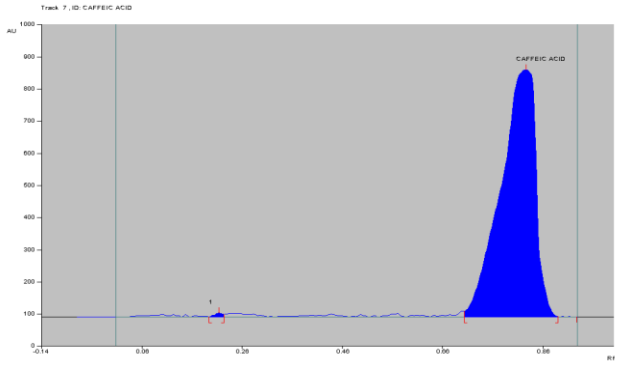


Fig- 8: Chromatogram of Caffeic Acid

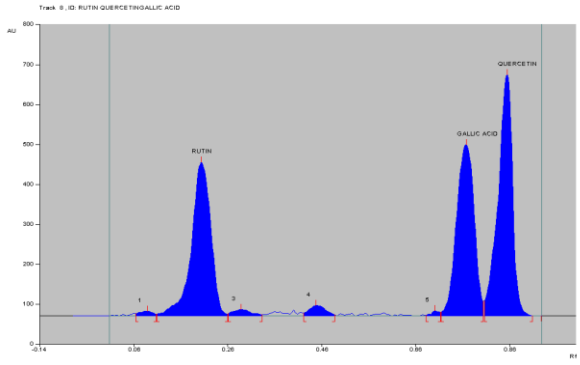


Fig- 9: Chromatogram of Rutin, Quercetin, Gallic Acid

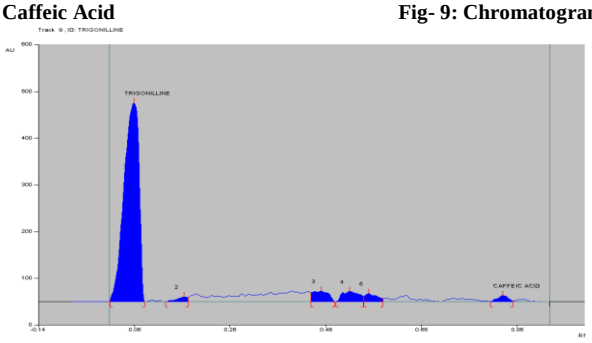


Fig- 10: Chromatogram of Trigonilline

Table 4: Estimation of free radical scavengers rutin, quercetin, gallic acid, caffeic acid and ferulic acid in herbal formulations

Track Number/Name of the formulation	Amount of sample applied in µl	R_f value of peak	Area of peak	Amount of marker present in applied µl of Sample in µg	Percentage of standard marker present in each 100mg of capsules
Track-1 MF_1	10.0 µl	0.20-rutin	1151.9	0.44 µg	0.04%
		0.79 Catechin	1434.9	0.54µg	0.05%
		0.82 Caffeic acid	2760.2	0.68 µg	0.08%
		0.85Quercetin	11879.6	3.95 µg	0.39%
Track-2 ORF	10.0 µl	0.77 Catechin	2188.2	0.82 µg	0.07%
		0.84-quercetin	8005.5	2.66 µg	0.22%
Track- MF_2 Phyllanthus	10.0 µl	0.05-trigonilline	109.2	0.05 µg	0.005%
		0.34-mangiferin	9471.2	3.04 µg	0.30%
Track-4 MF_3	10.0 µl	0.06-trigonilline	663.6	3.01 µg	0.30%
		0.77-gallic acid	1434.9	0.54 µg	0.05%
		0.86-quercetin	11013.0	3.66 µg	0.36%
Track-5 MF_4	10.0 µl	0.77-catechin	13305.9	5.06 µg	0.50%
		0.85-quercetin	11879.6	3.95 µg	0.39%
Track-6 Catechin-mangiferin	10.0 µl	0.34-mangiferin,	15535.3	5 µg	---
		0.82-catechin	8253.7	5 µg	---
Track-7caffeic acid	10.0 µl	0.82-caffeicacid	44033.5	5 µg	---
Track-8rutin ,gallic acid, quercetin	15.0µl	0.20-rutin,	12865.6	5 µg	---
		0.77-gallic acid	13141.0	5 µg	---
		0.85-quercetin,	15022.8	5 µg	---
Track-9 trigonilline	10.0 µl	0.06-trigonilline,	10901.3	5 µg	---

CONCLUSION:

The HPTLC of MF_1 was found to contain rutin 0.04% catechin 0.05% caffeic acid 0.08% and quercetin 0.39%. ORF capsules contains catechin 0.07% Quercetin 0.22%,MF_2 contains trigonilline 0.005% and mangiferin 0.3% MF_3 contains trigonilline 0.3%,gallic acid 0.05%, and Quercetin0.36%. MF_4 catechin 0.5% and Quercetin 0.39%, using the mobile phase Toluene: ethyl acetate: formic acid: methanol. Hence in this effort we estimated the well-known free radical scavenger's rutin, quercetin,catechin , caffeicacid, trigonillin and gallic acid in marketed herbal hepatoprotective formulations by HPTLC methods. This paper exposed that the levels of markers in formulations responsible for therapeutic activity.

REFERENCES:

1.Saleem TM, CM. Chetty, S. Ramkanth, V.S.T. Rajan, K.M. Kumar, K. Gauthaman, Hepatoprotective herbs—a review, Int. J. Res. Pharm. Sci, 2010; 1: 1–5.

2.GrattaglianoIL,BonfrateCV,DiogoHH,Wang,DQH,. Wang,P.Portincasa.Biochemical mechanisms in drug-induced liver in ury: certain ties and doubts. World J.Gastroenterol, 2009; 15: 4865–76.

3. AlshawshM.A.Abdulla, S.Ismail, Z.A Amin..2011 Hepatoprotective effectsof orthosiphon staminus extract on thio acetamide-induced liver Cirrhosis in rats. Evid. Based Complement. Altern. Med. Volume 2011, Article ID 103039, 6 pages doi:10.1155/2011/103039 Available at http://dx.doi.org/10.1155/2011/103039 [Accessed on 06 october 2016]

4. Spencer PSHH, Schaumberg AC. Ludolph. 2000. Experimental and Clinical Neurotoxicolog. second edition, New York, Oxford, Oxford University Press..

5.Muralidharan S, Dhananjayan V, Jayanthi P. Organochlorine pesticides in commercial marine fishes of Coimbatore, India and their suitability for human consumption. Environ. Res, 2009;109: 15–21

6.Aulakh RS, Bedi JPS, Gill BS, Joia PA, Pooni Sharma JK..Occurrence of DDT and HCH insecticide residues in human biopsy adipos tissues in Punjab, India, Bull. Environ. Contam.Toxicol. 2007; 78: 330–34.

7.Malarvannan G, Kunisue T, Isobe T, Sudaryanto A, Takahashi S, Prudente M, Subramanian A, Tanabe S Organohalogen compounds in human breast milk from mothers living in Payatas and Malate, the Philippines: levels, accumulation kinetics and infant health risk. Environ. Pollut, 2009; 157:1924–32.

8.Harbison RD, Bourgeois MM, Johnson GT.1998. Industrial Toxicology.414–28. Available at http://www.franklisitano- store.com/hamilton-and-hardy-s-industrial-toxicology.pdf. [Accessed on 11 november 2017)

9.Kostka GJ, Kope_c-Szlezak D, Palu.T. Early hepatic changes induced in rats by two hepatocarcinogenic organo halogen pesticides: bromopropylate and DDT. Carcinogenesis, 1996;17: 407–12.

10.Ben Rhouma KO, Tébourbi M. Sakly. Acute hepatotoxicity of DDT: effect on glucocorticoid receptors and serum transcortin. Indian J. Exp. Biol, 2000; 38: 452–56.

11.Barros SR, Pimente K, Simizu L, Azzalis I, Costa V.Junqueira. Dose-dependent study of liver lipid peroxidation related parameters in rats treated with pp'-DDT, Toxicol. Lett, 1994; 70: 33–38.

12.Marouani ND, Hallegue MS, Sakly Benkhalifa M, Ben Rhouma K., Tebourbi O. Adverse haemato biochemical effects of chlorinated insecticide in adult male rats, Int. J. Adv. Res, 2016; 959–67.

13.Harada T, Takeda M, Kojima S, Tomiyama N. 2016 Toxicity and carcinogenicity of dichlorodiphenyltrichloroethane (DDT), Toxicol. Res, 2016; 32: 21–33. Available on http://dx.doi.org/10.5487/TR.2016.32.1.021. [Accessed on 12 December 2016]

14.Chiba IA, Sakakibara TH. Iwata M, Ishizuka S, Tanabe F Akahori, Kazusaka A, Fujita S., Hepatic microsomal cytochrome p450s and chlorinated hydrocarbons in largha and ribbon seals from Hokkaido, Japan: differential response of seal species to Ah receptor agonist exposure, Environ. Toxicol. Chem 2002; 21: 794–806.

15.Harada TS, Yamaguchi R, Ohtsuka M, Takeda H, Fujisawa T, Yoshida, Enomoto A, Chiba Y, Fukumori S, Kojima N, Tomiyama Saka M, Ozaki M, Maita K, Mechanisms of promotion and progression of preneoplastic lesions in hepatocarcinogenesis by DDT in F344 rats, Toxicol. Pathol 2003; 31:87–98.

16.Jin X M, Chen LS, Li H, Li Z. The evaluation of-DDT exposure on cell adhesion of hepatocellular carcinoma, Toxicology, 2014; 322: 99–108.

17.Tebourbi O, Rhouma KB, Sakly M. DDT induces apoptosis in rat thymocytes, Bull. Environ. Contam.Toxicol, 1998; 61:216–223.

18.Jin X L, Song X, Liu M, Chen Z, Li L, Cheng H R. Protective efficacy of vitamins C and E on DDT-induced cytotoxicity via the ROS-mediated mitochondrial pathway and NF-kB/FasL pathway, PLoS One 2014;9. e113257, Available on http://dx.doi.org/10. 1371/journal .pone. 0113257

Barros et al., 1994, Marouani et al., 2016 and Harada et al., 2016.
19. Pérez-Maldonado IN, Herrera C, Batres LE, González-Amaro R, Díaz- Barriga F, Yáñez L. DDT-induced oxidative damage in human blood mononuclear cells, Environ. Res. 2005;98 :177–184.
20.Song QP, Zheng L, Qiu X, Jiang H, Zhao H, Zhou Q, Han X Diao. Toxic effects of male Pernaviridis gonad exposed to BaP, DDT and their mixture: a metabolomic and proteomic study of the underlying mechanism, Toxicol. Lett. 2016; 240:185–195.
21.Canales-Aguirre A E, Padilla-Camberos U, Gómez-Pinedo H, Salado-Ponce A, Feria-Velasco R, De Celis. Genotoxic effect of chronic exposure to DDT on lymphocytes, oral mucosa and breast cells of female rats, Int. J. Environ. Res. Publ. Health 2011; 8:540–553.
22.Huang BX, Ban J, He J, Tong J, Tian Y Wang. Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (Nelumbo nucifera Gaertn.) leaves, 2010Food Chem. 120 ; 873–78.
23. Ramasamy Arivukkarasu, Aiyalu Rajasekaran . Detection of Flavonoids, Phenolic Acids and Xanthones in Commercial Herbal Formulations by HPTLC Technique. Research Journal of Pharmacognosy and Phytochemistry. 2015,7(1):9-24.