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Original Research Article

# Detection and estimation of well-known free radical scavengers rutin, quercetin and gallic acid in market herbal anti-inflammatory and anti-arthritis formulations by HPTLC Methods

**Arivukkarasu R*[1], Rajasekaran A[1], Sethuramani A[2], Kattu raja S[2], Mohamed Ali Jinna A[2], Nandhini P[2], Ramya M Vinothini R[2]**

[1]KMCH College of Pharmacy, Coimbatore, Tamilnadu, India-641048
[2]Madurai medical college, Madurai, Tamilnadu, India-625020

***Corresponding author**
R. Arivukkarasu
Email: arivupriya@yahoo.co.uk

**Abstract:** The prime aim of the swot up is to observe the flavonoids and phenolic acids in three commercial herbal anti-arthritis formulations. HPTLC method was adopted to confirm the presence of these common secondary metabolites in the tested commercial herbal formulations. Results of the study clearly revealed that these three formulations contain flavonoids -quercetin rutin and phenolic acid category gallic acid. The developed HPTLC method can be employed for the routine investigations of well-known free radical scavenger's rutin, quercetin and gallic acid in marketed herbal anti-inflammatory and anti-arthritis formulations.
**Keywords:** Anti-arthritic, Rutin, Quercetin, Gallic acid, Herbal formulations, HPTLC.

## INTRODUCTION:

Standardization of herbal formulation in stipulations of raw materials, manufacturing practices and composition is important to guarantee quality and most favourable level of active principles for their bio effectiveness [1]. Identification of major and unique compounds in herbs as markers and development of analytical methodologies for monitoring them are the key steps involved in marker based standardization. High performance thin layer chromatography (HPTLC) is a preferred analytical tool for fingerprints and quantification of marker compounds in herbal drugs due to its simplicity, high sensitivity, accuracy and less expensive [2]. Harish *et al.;* [3] and Duthie *et al.;* [4] reported that rutin scavenges free radicals; Middleton [5] revealed that rutin suppresses cellular immunity as well as anti-inflammatory effect by Guardia [6]. More recent reports show that rutin also has antimicrobial activity [7]. Specifically Rotelli *et al.;* regarding this anti-inflammatory activity [8] the investigators determined such activity in an experimental model of adjuvant-carrageenan-induced inflammation in rats. Until now, however, the anti-inflammatory effect of rutin on inflammation caused by specific infectious diseases has not been determined. This information led Yongmoon Han [9] to investigate the effect of rutin on septic arthritis caused by *Candia albicans*. Furthermore, they determined the anti-arthritic mechanism by which rutin inhibits NO production by macro-phage, T-lymphocyte proliferation, and the growth of the fungus - a critical step in the pathogenesis of septic arthritis. Boyce [10] Van den Broek *et al.;* [11] Anderson *et al.;* and Zafi rova *et al.;* [12–13]. Recently, quercetin is shown to be effective in controlling rheumatoid arthritis Mamani-Matsuda [14] Kandere-Grzybowska [15] Tiku *et al.;* [16]. Quercetin has been reported to inhibit the allergic and inflammatory responses of the immune system Jackson *et al.;* [17] by modulating several aspects of cell function relevant to inflammatory arthritis. At the molecular level, quercetin is known to inhibit nuclear factor kappa B (NF-kB), a central transcription factor in flammatory and proliferative diseases Min *et al.;* [18]. Quercetin inhibits inflammatory aspects of synovial cell function, neutrophil activation and hence quercetin could be an effective anti-arthritic agent. No HPTLC method is reported in the literature for detection and estimation of well-known free radical scavengers rutin, quercetin and gallic acid in market herbal anti-inflammatory, anti-arthritis formulations and hence this paper describes the same.

## MATERIALS AND METHODS:

### Collection of herbal formulations for HPTLC screening:

Four formulations were procured from the market, all are capsule dosage form. The four market formulations were Arthrum capsules, Rumafort capsules, Arjit forte, and Rumawin. The ingredients of all commercial herbal formulations are provided in Table 1. Organoleptic evaluation of formulations were carried out and tabulated in Table 2.

**Table 1: Ingredients of all commercial herbal formulations**

| S.No | Rumafort Capsules | Arjit Forte Capsules | Arthrum Capsules | Rumawin Capsules |
|---|---|---|---|---|
| 1 | *Commiphora mukul* | Godanthibhaama | *Boswella serrata* | Shallai kukkul |
| 2 | *Rubia cardifolia* | Maha Yoharaja gukkulu | *Yogaraj guggula* | Kishore gukkul |
| 3 | *Dasamoolam* | Maha rasnadiquatha Choorna | *Pluchea lanceola* | Shuadishilajeet |
| 4 | *Alpinia galangal* | Shankhabhasma | *Vitex nigundo* | Nirgundi |
| 5 | *Zingiber officinale* | | | Maha rasnadi Ghana |
| 6 | *Apium graveolens* | | | Asgandh |
| 7 | *Vitex negundo* | | | Suranjian |
| 8 | *Strychnos nux-vomica* | | | Kuchlashudh |
| 9 | | | | Trifla |
| 10 | | | | Hardi |
| 11 | | | | Pipalli |
| 12 | | | | Gulancha |
| 13 | | | | Sundh |

**Table 2: Organoleptic evaluation and pH of formulations**

| Name of the formulation | Colour | Odour | Nature of particles | Taste | pH of the 1% solution of formulation |
|---|---|---|---|---|---|
| Arthrum capsules | Light whitish Brown | Aromatic | Fine powder | Aromatic taste | 6.2 |
| Rumafort capsules | Green | leafy | Fine powder | Pleasant Leafy | 5.7 |
| Arjit forte capsules | Green | Leafy | Fine powder | Bitter | 6.9 |
| Rumawin capsules | Green | leafy | fine powder | Bitter | 6.8 |

**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 and gallic acid 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 standards and extracts from the commercial herbal formulations:**

One gram of the each formulation was taken and sonicated with 10 ml of methanol. Sonicated and filtered and the filtrate solution was used for HPTLC analysis. Standard marker compounds were prepared using methanol to get a concentration 1 mg/1ml.Therefore 1000 µg of standard marker was dissolved in 1000 µl of methanol and 100mg of the capsule powder was dissolved in 1000 µl of methanol after filtration by whatmann filter paper for detection and estimation.

**Application of sample**:

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

**Development:**

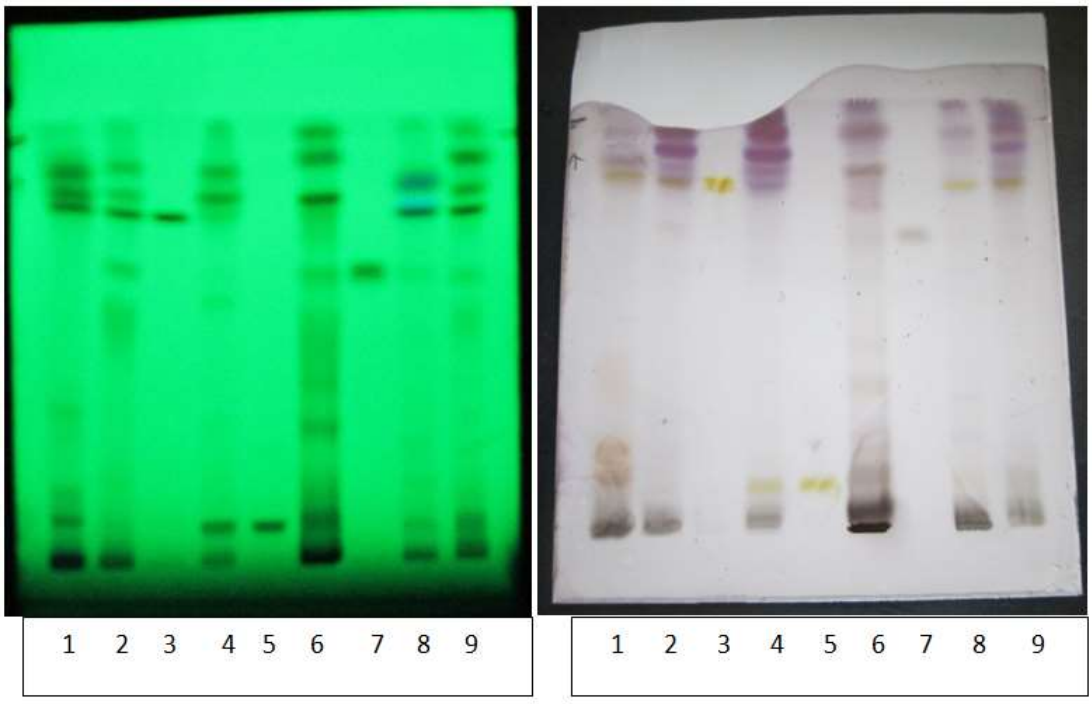
The chromatogram was developed in Camag glass twin -through chamber (10-10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid: methanol [3:6:1.6:0.4] for 10 min (temperature 25.°C, relative humidity 40%). The migration distance was 80 mm. TLC plates were air dried with air dryer. Densitometry scanning was performed using Camag TLC Scanner -III at 254 nm and 366 nm operated by a Wincat software [19].

**Detection:**

The plate was scanned at UV 254 nm using Camag TLC Scanner-3. $R_f$ value of each compound which were separated on plate and data of peak area of each band was recorded.

**RESULTS:**

The following different solvent compositions were tried for monitoring the elution of components in herbal formulations [20]. Ethyl acetate: glacial acetic acid formic acid: water (100:3:3:28), Ethyl Acetate: Methanol: Water Toluene (100:13:10:13), Chloroform: ethyl acetate: methanol (6:4:0.3). Ethyl Acetate: Methanol :Water Toluene (100:15.5:13.5:2),Ethyl acetate: methanol: water (100:15.5:13.5),Toluene :ethyl acetate :formic acid :methanol (3:6:1.6:0.4), Ethyl acetate: methanol : water(100:13.5:10),Toluene : ethyl acetate (93:7).Totally 8 mobile phase were trailed for better elution of formulations. Of which Toluene: ethyl acetate: formic acid: methanol (3:6:1.6:0.4) were given better elution for all the formulation to screen in one plate. The optimized chamber saturation time for mobile phase was 3.0 min at room temperature (25 ± 1°C). The densitometry analysis was performed at 254 nm in reflectance mode. The elution of all the formulation were carried out in mobile phase of toluene: ethyl acetate: formic acid: methanol (3:6:1.6: 0.4) and in this mobile phase elution was good results were tabulated by considering each $R_f$ value for one ingredients of formulation whether it may be pharmacologically active or inert but for screening the number of principle in the formulation can be considered as one of the principle in it. Therefore the obtained $R_f$ value were compared with $R_f$ value of the standard and well-known free radical scavengers rutin, quercetin and gallic acid in market herbal anti-inflammatory and anti-arthritis formulations (Table : 3). For identifying these free radical scavengers rutin, quercetin and gallic acid, we used UV light at 254 nm.




1. Herbal formulations, 2. Arthrum capsules, 3.Quercetin standard, 4.Rumafort capsules, 5.Rutin standard, 6.Arjit forte, 7.Gallic Acid standard, 8.Herbal formulations, 9.Rumawin

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

**Table 3: Rf values of free radical scavengers rutin, quercetin and gallic acid in herbal formulations**

| Track Number/Name of the formulation | Amount of Sample applied in µl | Number of peak | No of compounds and its $R_f$ values in Herbal marketed formulations | Name of the marker present in formulations |
|---|---|---|---|---|
| Track-1- Herbal formulations | 10 | 10 | **0.10**,0.17 0.32, 0.36 0.42 0.48,0.56 0.73,**0.83** and 0.86 | Rutin and quercetin |
| Track-2 Arthrum capsules | 10 | 9 | **0.09**,0.19,0.22,0.35,0.57,**0.69**,0.75, **0.82**,0.86 | Rutin, Gallic acid and quercetin |
| Track-3 Quercetin standard | 5 | 1 | **0.81** | Quercetin |
| Track-4 Rumafort | 10 | 09 | **0.09**,0.14,0.19,0.21,0.26,0.33,0.60, | Rutin and quercetin |

| | | | | |
|---|---|---|---|---|
| capsules | | | 0.67,**0.80** and 0.84 | |
| Track-5 Rutin standard | 5 | 1 | **0.08** | Rutin |
| Track-6 Arjit forte | 10 | 09 | **0.10**,0.15,0.18,0.31,0.41,0.50,0.67, **0.80**, and 0.84 | Rutin and quercetin |
| Track-7 Gallic Acid standard | 5 | 1 | **0.67** | Gallic acid |
| Track-8 Herbal formulations | 10 | 10 | **0.08**,0.13,0.18,0.24,0.27,0.35,0.51, 0.66,**0.80** and 0.87 | Rutin and quercetin |
| Track-9 Rumawin | 10 | 11 | **0.09**,0.15,0.18,0.25,0.27,0.33,0.41, 0.56, 0.66, 0.77, **0.81** and 0.86 | Rutin and quercetin |

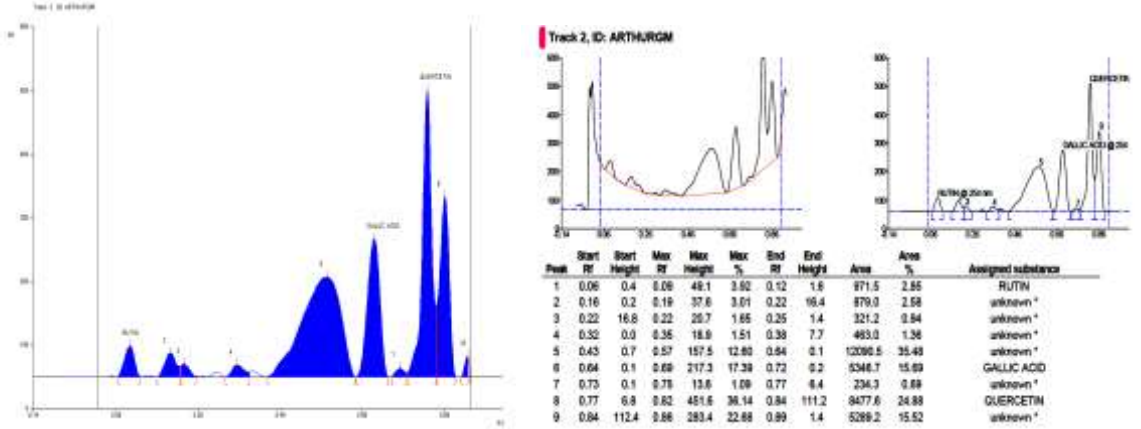



**Fig 2: Chromatogram of Arthrum capsules track-2**

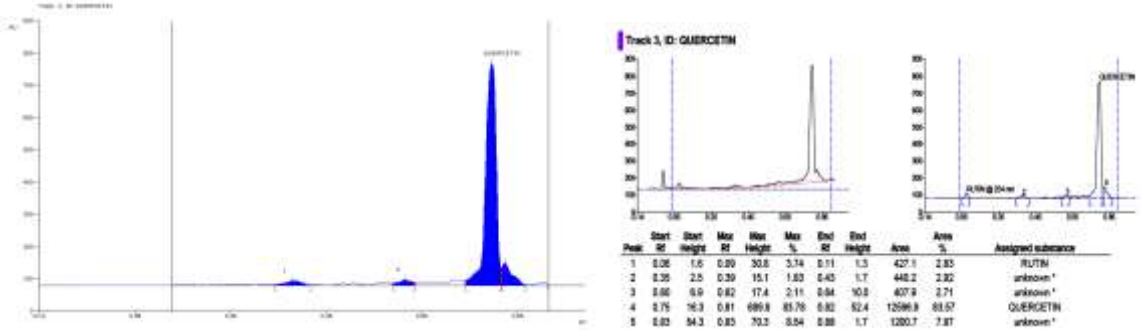
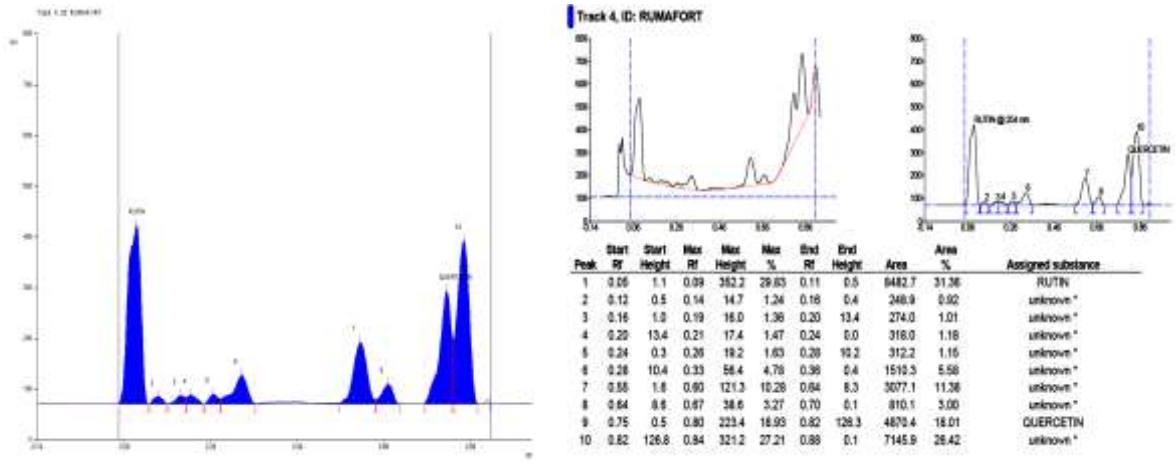
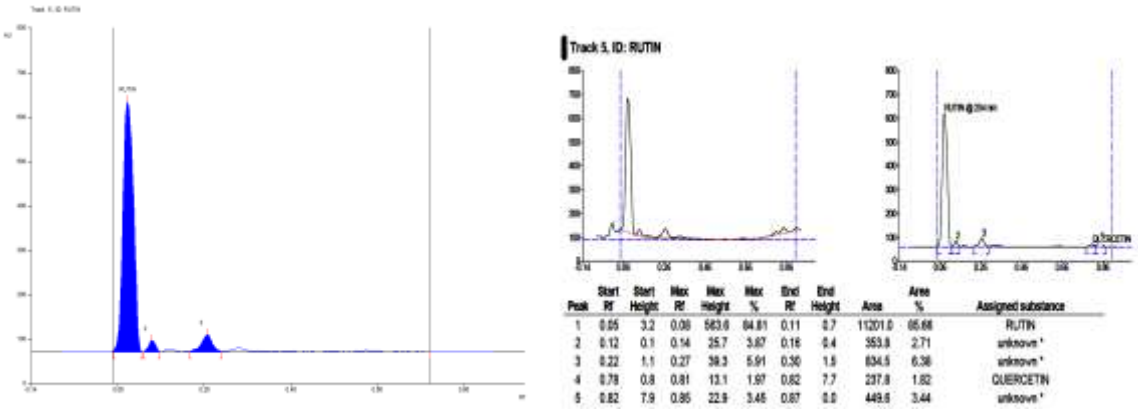



**Fig 3: Chromatogram of Quercetin track-3**



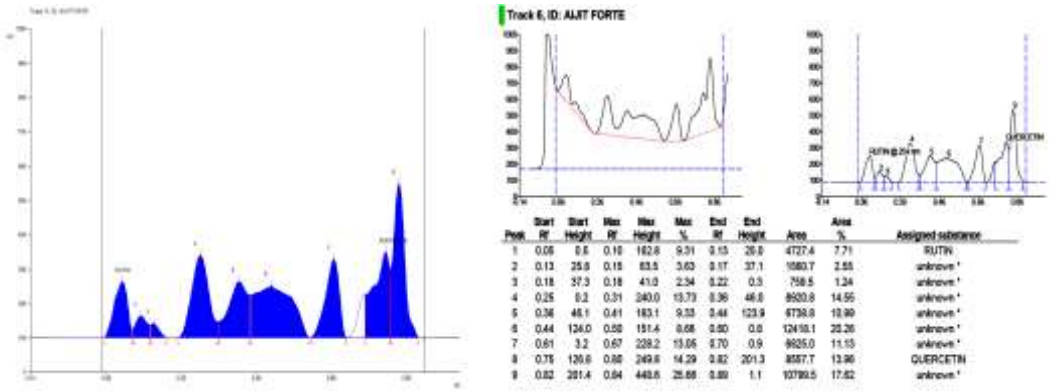

| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 1.1 | 0.09 | 352.2 | 29.63 | 0.11 | 0.5 | 8482.7 | 31.36 | RUTIN |
| 2 | 0.12 | 0.5 | 0.14 | 14.7 | 1.24 | 0.16 | 0.4 | 248.9 | 0.92 | unknown * |
| 3 | 0.16 | 1.0 | 0.19 | 16.0 | 1.36 | 0.20 | 13.4 | 274.0 | 1.01 | unknown * |
| 4 | 0.20 | 13.4 | 0.21 | 17.4 | 1.47 | 0.24 | 0.0 | 316.0 | 1.16 | unknown * |
| 5 | 0.24 | 0.3 | 0.26 | 19.2 | 1.63 | 0.28 | 10.2 | 312.2 | 1.15 | unknown * |
| 6 | 0.28 | 10.4 | 0.33 | 56.4 | 4.76 | 0.36 | 0.4 | 1510.3 | 5.58 | unknown * |
| 7 | 0.55 | 1.6 | 0.60 | 121.3 | 10.26 | 0.64 | 6.3 | 3077.1 | 11.36 | unknown * |
| 8 | 0.64 | 6.6 | 0.67 | 38.6 | 3.27 | 0.70 | 0.1 | 810.1 | 3.00 | unknown * |
| 9 | 0.75 | 0.5 | 0.80 | 223.4 | 18.93 | 0.82 | 128.3 | 4870.4 | 18.01 | QUERCETIN |
| 10 | 0.82 | 126.8 | 0.84 | 321.2 | 27.21 | 0.88 | 0.1 | 7145.9 | 26.42 | unknown * |

**Fig 4: Chromatogram of Rumafort capsules track-4**




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 3.2 | 0.09 | 563.6 | 84.81 | 0.11 | 0.7 | 11201.0 | 85.68 | RUTIN |
| 2 | 0.12 | 0.1 | 0.14 | 25.7 | 3.87 | 0.16 | 0.4 | 353.8 | 2.71 | unknown * |
| 3 | 0.22 | 1.1 | 0.27 | 39.3 | 5.91 | 0.30 | 1.5 | 834.5 | 6.38 | unknown * |
| 4 | 0.78 | 0.8 | 0.81 | 13.1 | 1.97 | 0.82 | 7.7 | 237.8 | 1.82 | QUERCETIN |
| 5 | 0.82 | 7.9 | 0.85 | 22.9 | 3.45 | 0.87 | 0.0 | 449.6 | 3.44 | unknown * |

**Fig 5: Chromatogram of Rutin track-5**




| Peak | Start Rf | Start Height | Max Rf | Max Height | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 0.6 | 0.10 | 162.6 | 9.31 | 0.13 | 26.0 | 4727.4 | 7.71 | RUTIN |
| 2 | 0.13 | 25.6 | 0.15 | 63.5 | 3.63 | 0.17 | 37.1 | 1580.7 | 2.58 | unknown * |
| 3 | 0.18 | 37.3 | 0.18 | 41.0 | 2.34 | 0.22 | 0.3 | 758.5 | 1.24 | unknown * |
| 4 | 0.25 | 0.2 | 0.31 | 240.0 | 13.73 | 0.36 | 46.6 | 8920.8 | 14.55 | unknown * |
| 5 | 0.36 | 46.1 | 0.41 | 163.1 | 9.33 | 0.44 | 123.9 | 6739.0 | 10.99 | unknown * |
| 6 | 0.44 | 124.0 | 0.50 | 151.4 | 8.66 | 0.60 | 0.6 | 12419.1 | 20.26 | unknown * |
| 7 | 0.61 | 3.2 | 0.67 | 228.2 | 13.06 | 0.70 | 0.9 | 6825.0 | 11.13 | unknown * |
| 8 | 0.75 | 126.6 | 0.80 | 249.6 | 14.29 | 0.82 | 201.3 | 8557.7 | 13.96 | QUERCETIN |
| 9 | 0.82 | 201.4 | 0.84 | 449.6 | 25.68 | 0.89 | 1.1 | 10799.5 | 17.62 | unknown * |

**Fig 6: Chromatogram of Arjit forte capsules track-6**

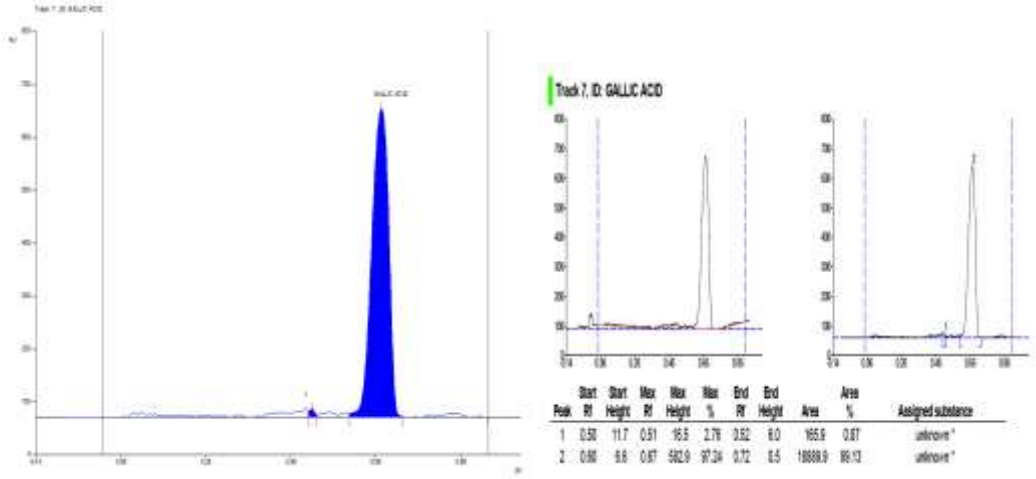



**Fig 7: Chromatogram of standard Gallic acid track- 7**

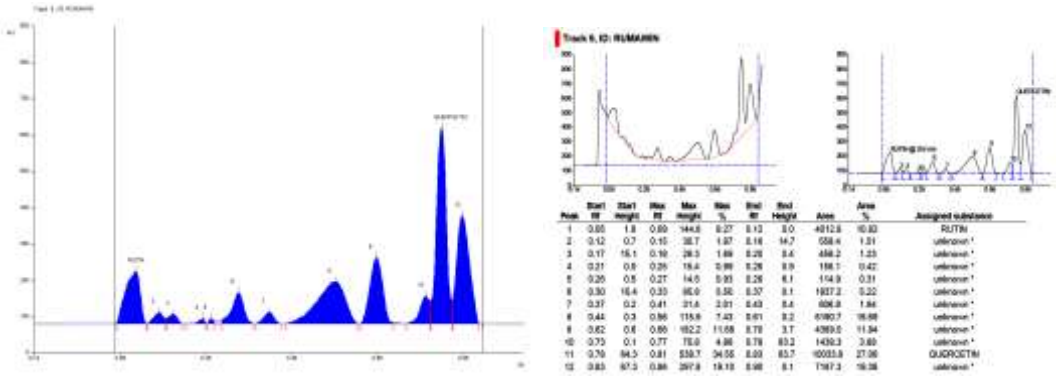


**Fig 8: Chromatogram of Rumawin track.-9**

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

| Track Number/Name of the formulation | Amount of Sample applied in µl | Rf 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-Herbal formulations | 10µl | 0.10-Rutin | 7848.6 | 3.50 µg | 3.50% |
| | | 0.83-Quercetin | 5702.3 | 2.26 µg | 2.26% |
| Track-2-Arthrum capsules | 10 µl | 0.09- Rutin | 0971.6 | 0.43 µg | 0.43% |
| | | 0.69-Gallic acid | 5346.7 | 1.41 µg | 1.41% |
| | | 0.82- Quercetin | 8477.6 | 3.36 µg | 3.36% |
| Track-3 Quercetin standard | 5 µl | 0.81- Quercetin | 12596.9 | 5.0 µg | 5.00% |
| Track-4 Rumafort capsules | 10 µl | 0.09- Rutin | 9482.7 | 4.23 µg | 4.23% |
| | | 0.80- Quercetin | 4870.4 | 1.93 µg | 1.93% |
| Track-5 Rutin standard | 5 µl | 0.08- Rutin | 11201.0 | 5.0 µg | 5.0% |
| Track-6 Arjit forte | 10 µl | 0.10- Rutin | 4727.4 | 2.11 µg | 2.11% |
| | | 0.80- Quercetin | 8557.7 | 3.39 µg | 3.39% |
| Track-7 Gallic Acid standard | 5 µl | 0.67- Gallic acid | 18889.9 | 5.0 µg | 5.00% |
| Track-8 Herbal formulations | 10 µl | 0.08- Rutin | 3100.9 | 1.38 µg | 1.38% |
| | | 0.80- Quercetin | 11232.0 | 4.45 µg | 4.45% |
| Track-9 Rumawin | 10 µl | 0.09- Rutin | 4012.8 | 1.79 µg | 1.79% |
| | | 0.81- Quercetin | 10035.8 | 3.98 µg | 3.98% |

**DISCUSSION:**

The HPTLC of Arthrum capsules found to contain rutin 0.43% gallic acid 1.41%and querctin3.36%. Rumafort capsules contains rutin 4.23% and quercetin 1.93%, Arjit forte contains rutin 2.11% and quercetin 3.39 %, and Rumawin contains rutin 1.79% and quercetin 3.98 %, using the mobile phase toluene: ethyl acetate: formic acid: methanol. Flavonoids and phenolic acids which serve as an important source of anti-oxidants found in different medicinal plants and related phytomedicines [21].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. These secondary metabolite 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 anti-inflammatory and anti-arthritis formulations by HPTLC methods. This paper exposed that the levels of markers in formulations responsible for theraputic activity.

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