1. Introduction

Euphorbiaceae family having 220 genera and 4000 plant species found in various tropical regions of India [1,2]. Following genera of Euphorbiaceae were reported as medicinal plants: Acalypha, Aleurites, Bridelia, Jatropha, Phyllanthus, Putranjiva, Ricinus [2–4]. Species of Euphorbiaceae family was commonly seen in India is Putranjiva roxburghii Wall which is known as child’s amulet tree or child-life tree [5]. Putranjiva is a mostly dioecious, evergreen tree, growing up to 18 m in height. The Putranjiva roxburghii Wall mentioned frequently recorded folk remedy claims in that the plant leaf, bark, seed, nuts are medicinally useful. Nuts are taken orally by women (sterile) in villages near Renuka forest division in Himachal to effect conception and attributed with the birth of child [5]. Bark and the seeds of this plants are useful in antitodal treatment of snake-bite. Its leaves and fruits, stones of this plant have been traditionally used for the treatment of fever, muscle twisting, aphrodisiac, arthralgia and rheumatism [6–8]. It was used as antinociceptive, antipyretic, anti-inflammatory, antioxidant [9].

Putranjiva roxburghii bark contained important phytoconstituents such as triterpenoids (putranjivanonol, putanjic acid, putranjivadione, roxburgholone), pentacyclic triterpene (friedelin, friedelanol) [10–12]. A friedelane triterpenoid keto acid (roxburghonic acid), biflavonoid (putraflavone) were isolated from the leaves part of Putranjiva roxburghii Wall and polyherbal formulations.

Putranjiva roxburghii Wall by HPTLC method [13]. Previous analytical work includes that amentoflavone, β-amyrin and stigmasterol determined from Putranjiva roxburghii Wall by HPTLC method [14,15]. So an attempt has been made to carry out detail chromatographic analysis of leaves and bark of Putranjiva roxburghii Wall.

In present study HPTLC method was developed and validated for the determination of friedelin in Putranjiva roxburghii Wall (family: Euphorbiaceae) leaf, bark extract and in polyherbal formulations. Analysis of samples were performed on TLC aluminium precoated plate (60 F254) by using mobile phase toluene: chloroform (9:1 v/v). Plate was derivatized with vanillin sulphuric acid and scanned at 580 nm. Developed method found to give compact spot for friedelin at Rf value 0.43 ± 0.01. The method was validated using International Council for Harmonization (ICH) guidelines including linearity, precision, accuracy, and robustness. Friedelin was found to be present in leaf extract of Putranjiva roxburghii Wall (0.003% w/w), in bark (0.04% w/w), formulation 1 (0.002% w/w) and formulation 2 (0.035% w/w). A good linearity relationship was found to be (100–500 ng spot−1) with correlation coefficient (r2) value of 0.9892 for friedelin. Limit of detection and limit of quantitation was found to be 32.15, 97.44 ng/band respectively for friedelin. The developed method was found to be accurate and precise with 0.78%, 0.9% (%RSD) for interday and intraday precision. Accuracy of the method was performed by recovery studies at three different concentration levels and the average percentage recovery was found to be 98.55% for friedelin. The proposed method for the quantitation of friedelin was found to be simple, specific, accurate and robust in Putranjiva roxburghii Wall and polyherbal formulations.

In the present study a validated HPTLC method for the quantification of friedelin in Putranjiva roxburghii Wall extracts and polyherbal formulations was developed. The developed method was found to be precise and accurate with RSD of 0.9% for interday and intraday precision. Accuracy of the method was performed by recovery studies at three different concentration levels and the average percentage recovery was found to be 98.55% for friedelin. The proposed method for the quantitation of friedelin was found to be simple, specific, accurate and robust in Putranjiva roxburghii Wall and polyherbal formulations.
Friedelin was pentacyclic triterpene isolated from several plant species such as *Terminalia avicennioides*, *Cissus quadrangularis*, *Acer manshuricum*, *Cassia siamea* Lam, *Celastrus vulcanicola*, *Maytenus aquifolium*, *Salacia campestris*, *Maytenus macrocarpa*, *Alandium salvi-folium* (Linn), *Holoptelea integrifolia* (Roxb) [16–22]. Structure of friedelin has shown in (Fig. 1). Friedelin compound shows effective ifolium (Linn), *mandshuricum*, *Cassia siamea* Lam, species such as and identification of friedelin in polyherbal formulation by high performance thin layer chromatography and this study also help in analysis of friedelin in other several plant species.

2. Materials and methods

2.1. Plant material

The leaves and trunk bark material of grown tree of *Putranjiva roxburghii* Wall was collected from Khadaki region of Maharashtra. The taxon was authenticated from Botanical Survey of India, Pune dated 18/08/2014 with Voucher number BSI/WRC/Cert./2014 and collection No. KKA 01. The herbarium specimen is deposited in the Modern college of pharmacy, Nigdi, Pune.

2.2. Chemicals and reagents

Friedelin was purchased from Sigma-Adrich (USA) (product code-101669048) all other solvents, reagents and precoated silica gel 60 F254 plates (HPTLC, 20 × 20 cm) were purchased from Merck (Germany).

Now a days in market we can get ayurvedic formulation Femiforte tablet (formulation 1) containing extracts of *Putranjiva roxburghii* (40 mg each tablet), Femiplex tablet (13.05 mg each tablet) (formulation 2) was procured from local market, Pune (Charak Pharma Pvt. Ltd. Baddhi, Dist Solan, Himachal Pradesh, India).

2.3. HPTLC instrumentation and experimental conditions

Method development parameters includes sample and test solution preparation, HPTLC instrumentation condition, preparation of developing chamber, derivatization reagents carried out as per guideline mention in United States Pharmacopeia (USP, Chapter, 203). According to this chromatographic analysis was done on pre-coated silica gel 60 F254 plates (10 × 10 cm with 200 µm thickness HPTLC). Samples of extracts, formulations and standards were applied by using microsyringe (Linomat syringe, Hamilton-Bonaduz switzerland, Camag, Switzerland) in band length 8 mm wide and 8 mm apart by Camag Linomat 5 sample applicator (Camag, Muttenz, Switzerland). The application rate of sample on plate was 150 nL⁻¹. The plate was developed in previously saturated 10 × 10 cm twin-trough glass chamber (Camag, Muttenz, Switzerland) at room temperature. Initially different mobile phases were used for chromatogram development from this best resolution was observed in the composition of Toluene: Chloroform (9:1 v/v) for friedelin. Dry thin layer chromatographic plate derivatized with vanillin sulphuric acid reagent, heat plate at 105 °C and observed separation of bands it helps in analysis of friedelin in leaf and bark Extracts and formulations [23]. Analysis carried out at 580 nm in absorbance remission mode by TLC scanner III (Camag, Muttenz, Switzerland) and win CATS version 1.4.0 software (Camag, Muttenz, Switzerland) were used in this study. Microsoft excel was also used to treat data statistically.

2.4. Preparation of standard solution

A stock solution of friedelin (100 µg/ml) was prepared by dissolving 10 mg of accurately weighed friedelin in 100 ml chloroform. For calibration 1–5 µl standard solution was applied to HPTLC plate in the range 100–500 ng per band.

2.5. Preparation of sample solution

Based on solubility of the marker compounds, chloroform extract leaf and bark were prepared by weighing 50 g of dried powdered of *Putranjiva roxburghii* and extraction was carried out by soxhlet extraction assembly for 6 h. Solution was filtered, concentrated and use for HPTLC analysis. From this weigh 10 mg leaf and bark extract transferred to a 10 ml volumetric flask. Chloroform was added to volumetric flask to make final concentration (1000 µg/ml).

Weigh five femiforte and ten femiplex tablets, crushed it, removed tablet coating and powdered were kept for maceration for overnight in 10 ml chloroform, filtered it and use for HPTLC analysis.

2.6. Method validation

The analytical method was validated for linearity, precision, accuracy, specificity, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Council for Harmonization (ICH, 2005) guidelines.

The linearity was carried out by applying different concentration of standard friedelin. Quantification’s of marker in samples were carried out by calibration curve. Limit of detection (LOD) and limit of quantitation (LOQ) were calculated based on standard deviation (SD) and slope (S) of the calibration curve at levels approaching to the LOD according to formula (LOD = 3.3(SD/S)) and LOQ = 10 (SD/S)). Precision studies include, repeatability and system precision. Accuracy by recovery studies were carried out by spiking known concentration of standard to pre-analyzed samples. The robustness was carried out by making small variation in optimized method parameters such as variation in composition of mobile phase, chamber saturation time etc. The specificity of the method was determined by comparing Rf values and ultraviolet-visible (UV) spectra of peaks of components in sample, formulation and standard chromatogram.

Fig. 1. Chemical structure of Friedelin.
3. Results and discussion

3.1. Solvent system optimization

For optimization of solvent system various compositions of mobile phases were use. When mobile phase consisting toluene: ethyl acetate: formic acid (9:1:0.1 v/v/v) component in samples not get resolved and Rp of standard was very close to solvent front, other composition of mobile as toluene: ethyl acetate (9:1 v/v) was not shows better resolution in peaks in samples. In order to improve resolution in between peaks mobile phase in composition of toluene: chloroform (9:1 v/v), gives compact peak of standard and standard in samples and formulations. Observation shows the same Rp value (Fig. 3) for friedelin in standard and samples.

3.2. Method validation

3.2.1. Linearity

For determining the linearity range of standard friedelin, a series of spots of different volumes (1, 2, 3, 4, 5 μl) were applied so as to get 100–500 ng quantity of standard per band, respectively. Linearity was evaluated in triplicate. The plate was scanned at 580 nm and curve was prepared with respect to area vs. amount per spot (Fig. 2). A good linearity relationship was found to be with correlation coefficient (r²) value of 0.9892 for friedelin (Table 1 and Fig. 2).

3.2.2. Quantification of friedelin in the leaves, bark and in polyherbal formulation containing Putranjiva roxburghii Wall

3 μl of the plant leaf, bark extract and 50 μl of formulation 1 and 2 were applied to HPTLC plate in triplicate and the amount of friedelin in samples were determined by using calibration curve of standard. Previously HPTLC determination of friedelin was reported in Alangium salvifolium (Linn), Holoptelea integrifolia (Roxb) [24]. This method shows the presence of 0.003% w/w for friedelin in leaf extract, 0.04% w/w in bark extract, 0.002% w/w in formulation 1 and 0.035% w/w in formulation 2.

3.2.3. Limit of detection and quantitation

In order to determine limit of detection (LOD) and limit of quantitation (LOQ) were calculated according to formula (LOD = 3.3(SD)/S) and LOQ = 10 (SD/S)) and found to be 32.15, 97.44 ng spot⁻¹ for friedelin (Table 1).

3.2.4. Precision

Precision studies were carried out to show the reproducibility of the proposed developed method. Intraday precision study was carried out by applying six times 300 ng per band of same concentration. It can be analyzing at three different times in a day for intraday precision and the same procedure was followed for three different days to determine interday precision. The results were reported as SD (%RSD) (Table 2). The %RSD was found to be 0.78%, 0.9% for interday and intraday precision. The low %RSD indicated the method is precise for the analysis (Table 2).

3.2.5. Specificity

The specificity of the method was determined by analysing standard drug and sample. The presence of friedelin in leaf, bark and formulations were confirmed by comparing Rp and ultraviolet–visible spectra of sample with standard. Purity of sample spot corresponding to friedelin in sample and both formulations were analyzed by superimposing the spectrum of standard and sample peaks (Fig.4).

3.2.6. Recovery studies (Accuracy)

Accuracy of method was studied by performing recovery studies at 3 levels of friedelin. The pre-analyzed samples were spiked with 80%, 100% and 120% of the standard friedelin and analyzed by the proposed HPTLC method. The experiment was conducted six times the percentage recovery at three different levels of friedelin was found to be 98.24, 98.59, 98.82% respectively (Table 3).

3.2.7. Robustness

Robustness was studied in triplicate at 300 ng band⁻¹ by making small variation in optimised method parameters such as variation in composition of mobile phase, chamber saturation time. The results were examined in terms of relative standard deviation (% RSD) and standard error of peak area (Table 4). Mobile phase prepared by solvent system such as Toluene: chloroform (9:1 v/v), (9.2:0.8 v/v), (8.8:1.2 v/v) etc. Duration of saturation time change during chromatograph development (18, 20 and 22 min) respectively. The plate was activated at 110 °C for 20 min and analyzed at 580 nm. By introducing small changes into TLC method % RSD was obtained less than 2% proved the robustness of proposed method.

4. Conclusion

In present study HPTLC method was developed and validated for the determination of friedelin in Putranjiva roxburghii Wall leaf, bark and in polyherbal formulation, which shows 0.003% w/w for friedelin in leaf extract, 0.04% w/w in bark extract, 0.002% w/w in formulation 1 and 0.035% w/w in formulation 2. The proposed
Method was found to be simple, accurate, specific and robust for the analysis of friedelin in crude drug sample and polyherbal formulations. So these studies shade on chromatographic analysis of Putranjiva roxburghii Wall and this plant containing polyherbal formulations. Based on these results bark of this plant contain higher friedelin as compare to leaf, so concentrated fractions or extract of bark of Putranjiva roxburghii Wall is a rich source of friedelin and may be more useful for formulations to treat female infertility.

Fig. 3. HPTLC Chromatogram of standard Friedelin (a), leaf extract (b), Bark extract (c), formulation 1 (Femiforte Tablet) (d), formulation 2 (Femiplex tablet) (e) at 580 nm.

Table 1
Method validation parameters for the quantitation of friedelin by HPTLC.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range of linearity (ng/band)</td>
<td>100–500</td>
</tr>
<tr>
<td>Regression of equation</td>
<td>Y = 1642.47 + 17.11x</td>
</tr>
<tr>
<td>Slope</td>
<td>17.11</td>
</tr>
<tr>
<td>Correlation coefficient (r²)</td>
<td>0.9892 ± 0.003</td>
</tr>
<tr>
<td>LOD (ng/band)</td>
<td>32.15 ng</td>
</tr>
<tr>
<td>LOQ (ng/band)</td>
<td>97.44 ng</td>
</tr>
</tbody>
</table>
Table 2
Interday and intraday precision of HPTLC.

<table>
<thead>
<tr>
<th>Amount (ng/band)</th>
<th>Interday precision</th>
<th>Intraday precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean area SD %RSD</td>
<td>Mean area SD %RSD</td>
</tr>
<tr>
<td>Friedelin 300</td>
<td>8123.43 63.85 0.78</td>
<td>7754.5 70.54 0.9</td>
</tr>
</tbody>
</table>

* Mean of six determinations.

Fig. 4. Overlay UV absorption spectra of Friedelin in peaks of standard and extracts (a), Friedelin in peaks of standard and formulations (b) at 580 nm.
Table 3
Results of accuracy study.

<table>
<thead>
<tr>
<th>Level of recovery (%)</th>
<th>Theoretical content (µg/band)</th>
<th>Experimental content (µg/band)</th>
<th>% RSD</th>
<th>% mean* recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>0.57</td>
<td>0.56 ± 0.005</td>
<td>0.89</td>
<td>98.24</td>
</tr>
<tr>
<td>100</td>
<td>0.71</td>
<td>0.70 ± 0.005</td>
<td>0.7</td>
<td>98.59</td>
</tr>
<tr>
<td>120</td>
<td>0.85</td>
<td>0.84 ± 0.005</td>
<td>0.58</td>
<td>98.82</td>
</tr>
</tbody>
</table>

* Mean of three determinations.

Table 4
Robustness study for the HPTLC method.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Chromatographic changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mobile phase composition</td>
<td>Level</td>
</tr>
<tr>
<td>9.2/0.8</td>
<td>±2</td>
</tr>
<tr>
<td>9:1</td>
<td>0</td>
</tr>
<tr>
<td>8.8:1.2</td>
<td>−2</td>
</tr>
<tr>
<td>Saturation time</td>
<td>(±10%)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean of three determinations.

Conflicts of interest
There are no conflicts of interest.

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