Chromatographic Determination of Allelochemicals (Phenolic Acids in \textit{Jatropha curcas} by HPTLC

S. Rejila$^1$, N. Vijayakumar$^1$ and M. Jayakumar$^2$

$^1$Research Center in Botany, S. T. Hindu College, Nagercoil, Tamilnadu, India  
$^2$Research Department of Botany, VHNSN College, Virudhunagar, Tamilnadu, India

ABSTRACT

Free and esterified phenolic acids of \textit{Jatropha curcas} were extracted with Methanol in soxhlet apparatus and determined by HPTLC. The phenolic acid such as kaempferol, coumarin, catechin, and quercetin acids were detected by the Linomat 5 (Camag, Switzerland). The Linomat 5 is controlled by wincats planar chromatography manager, version 1.3.4. Plate dimensions, number and distance of tracks, names of samples and volumes to be applied on to each track are conveniently programmed and saved as wincats analysis file. The planar chromatogram was developed with Toluene-Acetone-Formic acid (4.5: 4.5: 1) and the developed plate were sprayed with respective spray reagent and dried at 100°C in Hot air oven. The plate was photo-documented at Day light and UV366nm using Photo-documentation (Camag reprostar 3) chamber.

Key words: \textit{Jatropha curcas}, Phenolic Acid, HPTLC, Allelochemicals.

INTRODUCTION

\textit{Jatropha} is a multi-purpose tree, growing naturally in countries of the equatorial America, whereas it has been spread to other tropical countries as well. \textit{Jatropha} seeds are rich in oil and when extracted, pure plant oil can be used directly or as biodiesel in engines. For this reason, \textit{Jatropha} is an attractive crop and it is being introduced rapidly in various rural programs, as it may contribute to rural development income by intercropping methods, generation and increasing the efficiency of rural and agricultural processes, throughout the world, including India they stared to cultivate the \textit{Jatropha} along with other crops. During the intercropping system \textit{Jatropha} showed allelopathy effect on nearby plants reported [1], this allelopathic effect may be due the presence of allelochemical. The allelochemicals like phenolic acid is present in all plants tissues including leaves, stems, flowers and roots, seeds and buds. These allelochemicals are usually called secondary plant products of the main metabolic pathways in plants [2]. They may be water-soluble substances that are released into the environment through leaching, root exudation, volatilization or decomposition of plant residues. Elemore [3, 4] have reported that the allelochemicals may released by seeds and leaves into the soil.

Phenolics are secondary metabolites derived from the aromatic amino acids synthesized through shikimic acid pathway [5]. Terrestrial and aquatic plants release several phenolics directly from their living tissues and/or indirectly after death and decomposion of the tissues [6]. These allelochemicals such a pelohic acid may be allelopathic effect on the growth of nearby plant by inhibiting or stimulating the seed germination and seedling growth [7, 8, 9]
Therefore, the aim of the present study is to determine the amount of allelochemicals like phenolic acid present in *Jatropha curcas* by HPTLC.

**MATERIALS AND METHODS**

**Plant Material**
Mature fresh leaves of *Jatropha curcas* were collected from Jatropha intercropping experimental field from S.T. Hindu College, Nagercoil, Tamilnadu, India. The collected leaves were dried in an oven at 60°C ± 2°C for four days powdered (40 mesh) and used for phenolic estimation.

**Estimation of Total Phenolics**
Total phenolic content of root was measured based on Folin-Ciocalteu assay [10]. Briefly, 1.2ml of sodium carbonate (7.5% w/v) was added to the 5gm of methanolic extract of root. After 30 min, absorbance was measured at 765nm with UV/Vis spectrophotometer (Elico, India). Total phenolic content was expressed as mg gallic acid equivalents (GAE)/g fresh weight.

**Preparation of extract**

**HPTLC Analysis for Phenolic Acids:** A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile. The 5gram of dried plant material of *Jatropha curcas* were extracted with methanol in soxhlet apparatus for 3hrs and then allowed to cool and filtered the content and concentrated using Vacuum flash evaporator. Dissolved the content with 1ml Methanol and centrifuged at 3000rpm for 5min. This solution was used as test solution for HPTLC analysis.

**RESULTS AND DISCUSSION**

**Estimation of Total Phenolics**
In the present study, we examined total phenolics (TF) using gallic acid as standard (R =0.9968), the content mlLG1). The mixtures were allowed to stand of total phenolics in methanol extract of *Jatropha curcas* leaf amounted to 35.24 ± 0.02 mg/g.

**HPTLC Fingerprinting Profile for Phenolic Acids**
HPTLC profile of methanol extract of *Jatropha curcas* leaf was recorded in Table 1. Blue, brown color zone was detected in UV after derivetaization in the chromatogram (Fig. 1 & 2) confirms the presence of phenolic acids. The extracts were run along with the standard phenolic compounds. The *Jatropha* leaf extracts which shows the presence of different types of phenolic acids in the chromatograph as well as in UV after derivatization. The Rf value of the leaf extract was found to be 0.04, 0.15, 0.23, 0.28, 0.67, 0.70, 0.75, 0.83, 0.90, 0.97 of peak 1, 2, 3, 4, 5, 6, 7, 8,9,10

---

*Pelagia Research Library*
respectively. Among them peaks 2, 3, 4, 5 were identified as a phenolic acid such as kaemferol, coumarin, catechin and quercetin compared with respective standards. In mean while rest of the peaks are designated as unidentified. These phenolic compounds may be exhibiting allelopathic effects in the Jatropha leaf extract. The peak height of the respective phenolic acid was also given in the Table 1.

**Fig: 1 Chromatogram before Derivatization**

![Chromatogram before Derivatization](image1)

*KML: Kaempferol, COU: Coumarin, CAT: Catechin, QUE: Quercetin, X5: Sample Code (Jatropha curcus Leaf)*

**Fig 2: Chromatogram after Derivatization**

![Chromatogram after Derivatization](image2)

*KML: Kaempferol, COU: Coumarin, CAT: Catechin, QUE: Quercetin, X5: Sample Code (Jatropha curcus Leaf)
Fig 3: HPTLC Chromatogram of Jatropha curcus leaf extract showed Peak densitogram display (Scanned at 254nm)

HPTLC Chromatogram of Standard Peak densitogram display (Scanned at 254nm)

Fig 3: Kaempferol  
Fig 4: Coumarin
The peak such as 2,3,4,5 Rf value was coinciding with standard (kaemferol, coumarin, catechin and quercetin) Rf value 0.15, 0.23, 0.28, 0.67 and its peak area was 6424.3, 9926.5, 6139.2, 11809.2 respectively. The results of this study infer that the Jatropha leaf showed the presence phenolic compound such as kaemferol, coumarin, catechin and quercetin. The allelopathic effect of Jatropha curcus on green chili and sesame was reported [9]. Therefore this allelopathic effect may be due to the presence of these allelochemicals.

Table 1: Peak table with Rf values, height and area of phenolic compounds and unknown compounds of Jatropha curcus leaf Methaol Extract

<table>
<thead>
<tr>
<th>Peak</th>
<th>Rf Value</th>
<th>Height of the Peak</th>
<th>Area of the Peak</th>
<th>Assigned substance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.04</td>
<td>177.8</td>
<td>2832.9</td>
<td>Unknown</td>
</tr>
<tr>
<td>2</td>
<td>0.15</td>
<td>174.1</td>
<td>6424.3</td>
<td>Kaemferol</td>
</tr>
<tr>
<td>3</td>
<td>0.23</td>
<td>283.1</td>
<td>9926.5</td>
<td>Coumarin</td>
</tr>
<tr>
<td>4</td>
<td>0.28</td>
<td>216.3</td>
<td>6139.2</td>
<td>Catechin</td>
</tr>
<tr>
<td>5</td>
<td>0.67</td>
<td>297.8</td>
<td>11809.2</td>
<td>Quercetin</td>
</tr>
<tr>
<td>6</td>
<td>0.70</td>
<td>205.4</td>
<td>4610.1</td>
<td>Unknown</td>
</tr>
<tr>
<td>7</td>
<td>0.75</td>
<td>51.8</td>
<td>1841.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>8</td>
<td>0.83</td>
<td>22.3</td>
<td>540.5</td>
<td>Unknown</td>
</tr>
<tr>
<td>9</td>
<td>0.90</td>
<td>23.2</td>
<td>616.8</td>
<td>Unknown</td>
</tr>
<tr>
<td>10</td>
<td>0.97</td>
<td>144.8</td>
<td>4285.2</td>
<td>Unknown</td>
</tr>
</tbody>
</table>

CONCLUSION

In the present study four different types of phenolic compounds (Allelochemicals) have been identified from methanolic extract of the Jatropha curcus leaf by HPTLC analysis. The presence of these various phenolic compounds may be stimulate the allelopathic acivity of Jatropha curcus on nearby crop plants in Jatropha intercropping system.

REFERENCES


Pelagia Research Library