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Chromatographic Determination of Allelochemicals (Phenolic Acids in *Jatropha curcas* by HPTLC

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ABSTRACT

Free and esterfied phenolic acids of 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: *Jatropha curcas*, Phenolic Acid**,** HPTLC, Allelochemicals.

INTRODUCTION

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. *Jatropha* seeds are rich in oil and when extracted, pure plant oil can be used directly or as biodiesel in engines. For this reason, *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 *Jatropha* along with other crops. During the intercropping system 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 allelochemical 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 decomposition of the tissues [6]. These allelochemicals such a peholic 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 curcus* 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 a $60^0C \pm 2^0c$ for four days powdered (40 mesh) and used for phenolic

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 5 gm 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 Phenoilc Acids: A densitometric HPTLC analysis was performed for the development of characteristic finger printing profile. The 5gram of dried plant material of *Jatropha curcus* 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.

Jatropha curcas leaf methanolic extract was dissolved with HPLC grade methanol 100 mg/0.5ml.The solutions was centrifuged at 3000 rpm for 5 min and used for HPTLC analysis. Then, 2 µl of the samples were loaded as 7 mm band length in the 10 x 10 Silica gel 60F TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturation with solvent vapor) with respective mobile phase (polyphenolic compound) and the plate was developed in the respective mobile phase (Toluene-Acetone-Formic acid 4.5:4.5:1) up to 90 mm. The developed plate was dried using hot air to evaporate solvents from the plate and sprayed with stannic chloride reagent. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV366 nm. Finally, the plate was fixed in scanner stage and scanned at 254nm. The Peak table, Peak display and Peak densitogram was identified [11].

The samples were spotted in the form of bands with Camag microlitre syringe on a pre-coated silica gel plates 60F 254 [10 cm X 10 cm with 0.2 mm thickness, E.Merck] using Camag linomat IV applicator. Automatic sample spotter of band width 7 mm. The plates were developed in a solvent system in CAMAG glass twin through chamber previously saturated with the solvent for 30 min. the distance was 8 cm. subsequent to the scanning, TLC plates were air dried and scanning was performed on a TLC Scanner. The TLC Scanner 3 (Camag, Switzerland) is controlled by wincats 1.3.4 version, that consisted of light sources as mercury vapour lamp (line spectrum 220 to 580 nm), Halogen-Tungsten lamp (continuum 350 to 800 nm) and Deuterium lamp (continuum 190 to 450 nm), Optical system consisted of 190 to 800 nm transmission that makes the measurement based on the density of the compound [12].

RESULTS AND DISCUSSION

Estimation of Total Phenolics

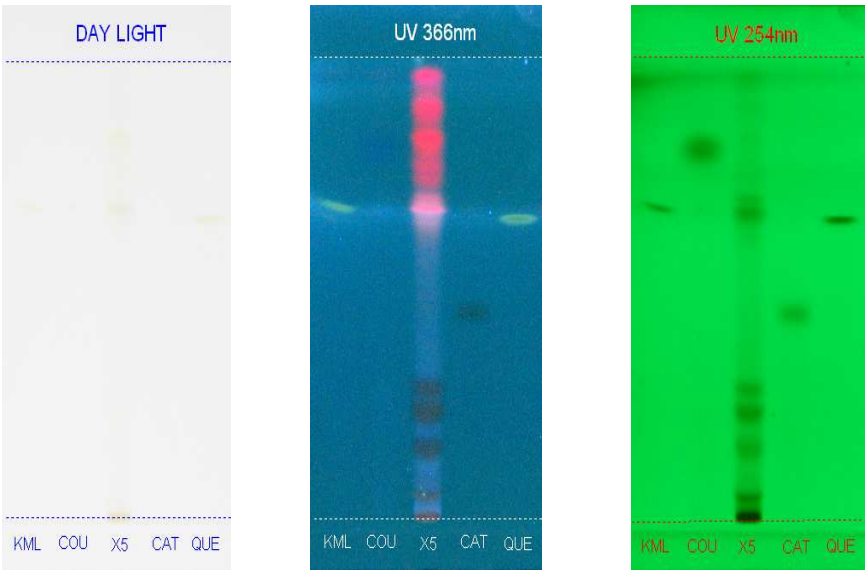
In the present study, we examined total phenolics (TF) using gallic acid as standard (R =0.9968), the content mLG1). The mixtures were allowed to stand of total phenolics in methanol extract of *Jatropha curcus* leaf amounted to 35.24 ± 0.02 mg/g.

HPTLC Fingerprinting Profile for Phenolic Acids

HPTLC profile of methanol extract of *Jatropha curcus* 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

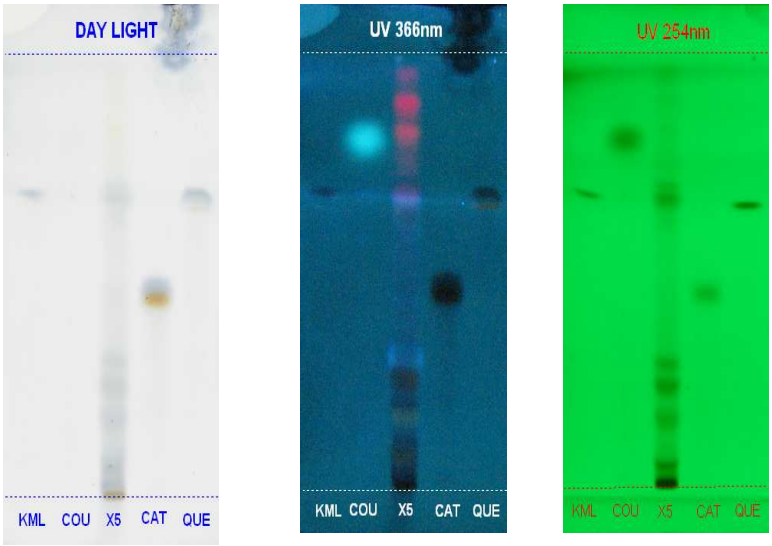
respectively. Among them peaks 2 3, 4, 5 were identified as a phenolic acid such as kaemferol, coumarin, catechin and quercrtin compared with respective standards. In mean while rest of the peaks are designated as unidentified. These phenolic compounds may be exhibiting allelopathic effects in the Jatropha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

Fig: 1 Chromatogram before Derivatization



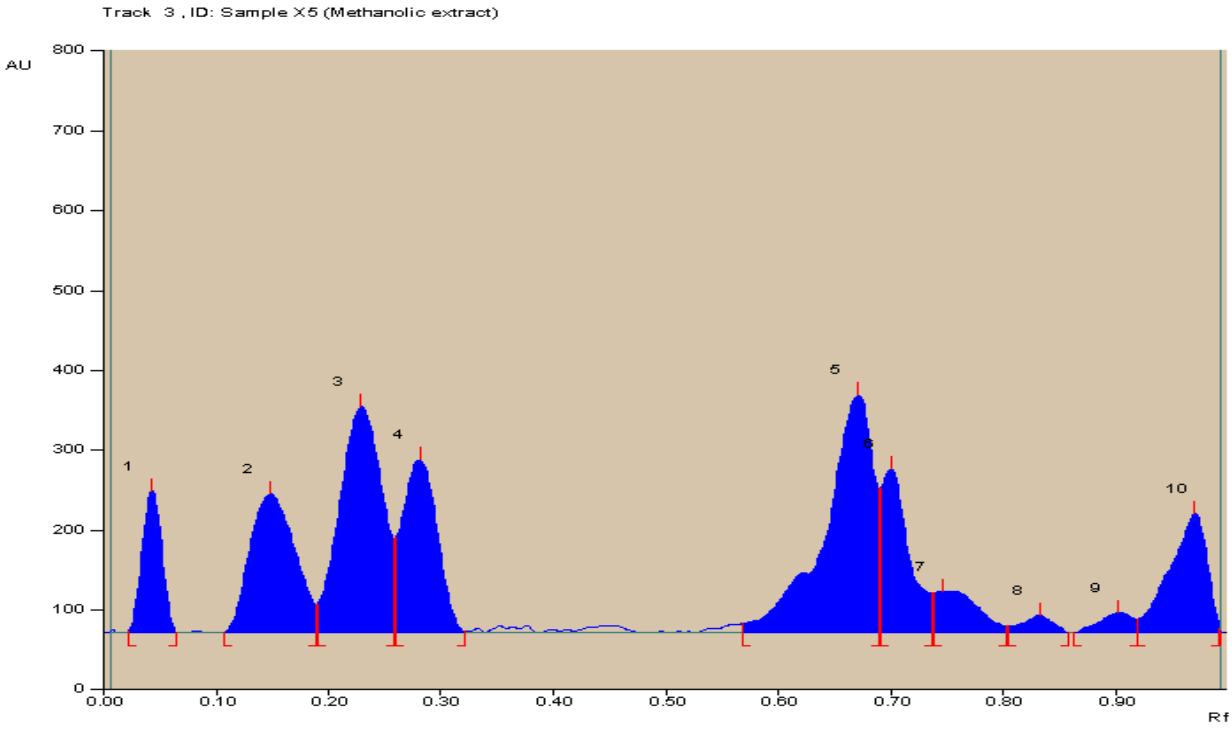
KML: Kaempferol, COU: Coumarin, CAT: Catechin, QUE: Quercetin, X5: Sample Code (Jatropha curcus Leaf)

Fig 2: Chromatogram after Derivatization



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

Fig 3: HPTLC Chromatogram of Jatropha curcus leaf extarct showed Peak densitogram display (Scanned at 254nm)



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

Fig 3: Kaempferol

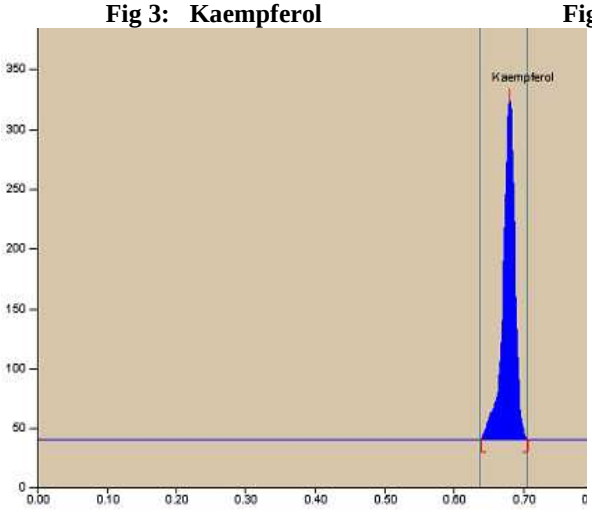


Fig 4: Coumarin

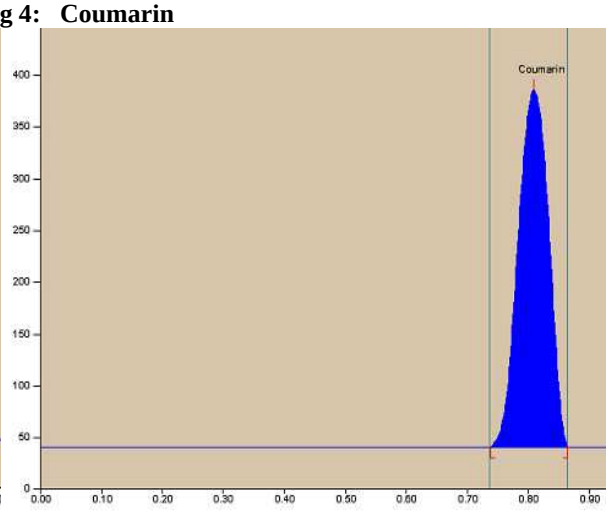


Fig 5: Catechin

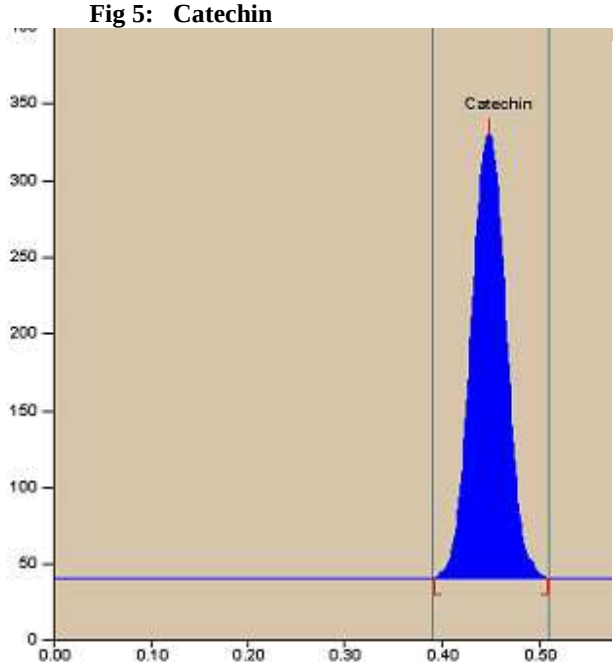
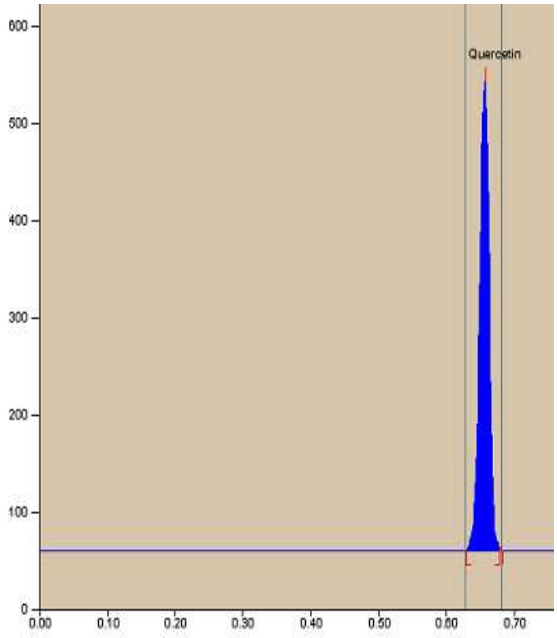


Fig 6: Quercetin



The peak such as 2,3,4,5 Rf value was coinciding with standard (keamferol, 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 keamferol, coumarin , catechin and quercetin. The allelopathic effect of Jatropha curcus on green chilli 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

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

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 phenoilc compounds may be stimulate the allelopathic acivity of *Jatropha curcus* on nearby crop plants in Jatropha intercropping system.

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