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# ISOLATION AND CHARACTERIZATION OF LEAF EXTRACT OF DERRIS TRIFOLIATA

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## ABSTRACT

Derris trifoliata belongs to the family Fabaceae of the order Papilionoideae. D. trifoliata is used in local medicine in India as a stimulant, antispasmodic and counter-irritant, and against rheumatism, chronic paralysis and dysmenorrhoea. The bioactive principle present in the leaves of Derris trifoliata was isolated and characterized in the present study. The powdered leaves were repeatedly extracted in methanol. The crude methanolic extract upon separation through silica gel column chromatography yielded 70 fractions which were then separated further by thin layer chromatography and isolated three different compounds. These fractions were further characterized by high performance thin layer chromatography and the $R_f$ values are identified accurately for individual peaks. This yields the complete phytochemical characterization of the methanol extracts of Derris trifoliata.The study attempts to identify the new compounds in this plant that revealed three compounds. The purity of the compounds was determined for the effective utilization of the compounds in the production of novel pharmaceutical preparations.

**KEYWORDS:** **Derris trifoliata, Methanol extract, Column chromatography, TLC, HPTLC.**

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# INTRODUCTION

The chemical constituents of the medicinal plants, particularly the secondary metabolites have pronounced pharmacological actions on animal system and organs. Several bioactive compounds were isolated from the plant sources such as digoxin, digitoxin, morphine, reserpine, taxol, vinblastine, vincristine and quercetin[1]. which has different pharmacological properties. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involve the use of plant extracts[2]. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents present in the plant play a significant role in the identification of crude drugs. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins and terpenoids[3].Derris trifoliata is one of the most commonly used insecticides. The leaves contain the chemical compound rotenone, a poison that kills a wide range of creatures from insects to earthworms and fish[4,5]. This poison is used in catching the fishes. The leaves of Derris trifoliata were proved to have many therapeutic uses, including stimulant, antispasmodic, counter-irritant, rheumatism, chronic paralysis, and dysmenorrhea. The decoction of roots is used against fever and internally against sores. The roots or stem is used as laxative, carminative and anti-arthritis agent[6].

# MATERIALS AND METHODS

### *Collection of Derris trifoliata*

Derris trifoliata leaves were collected at random from Rameshwaram Mangrove forests (Tamilnadu), India.

### *Preparation of the Plant Materials*

The leaves were dried in the shade for 7 days at room temperature (28 ±2°C) and ground to a fine powder using Grinder IKA®-WERKE, IKA MF10 Machine and sieved through a 0.25 µm mesh. The powder samples were kept at room temperature in a covered glass container to protect them from humidity and light prior to extraction.

### *Extraction of Derris trifoliata Leaves Extracts*

50g dried powder leaves were exhaustively extracted by macerated in 2.0 L methanol solvent for 2 days at room temperature (28±2°C). The solvent-containing extract was then decanted and filtered by vacuum filtration (GAST, DOA-P504-BN, USA). The extraction of the ground leaves was further repeated (twice) with methanol (1.5 L each time). The filtrate from each extraction was combined and the excess solvent was evaporated under reduced pressure at 40°C using a rotary evaporator (Heidolph-instruments, Rotavapor, Germany) to give concentrated crude methanolic extracts, dried in oven at 50°C to give dark green extracts. The weights of all the extracts were measured after solvent evaporation and then kept into a glass container prior to use.

### *Chemical characterization of leaf extract of Derris trifoliata*

#### *I By Column Chromatography*

Solvent-solvent partitioning (Fig I) was done by using the protocol designed by Kupchan and Tsou[7,8]. The crude extract (5 gm) was triturated with 90% methanol. The prepared solution was then fractionated successively using solvents of increasing polarity, such as hexane and ethyl acetate. Both the fractions were evaporated to dryness by using a rotary evaporator at low temperature of 39°C and kept in air tight containers for further analysis.

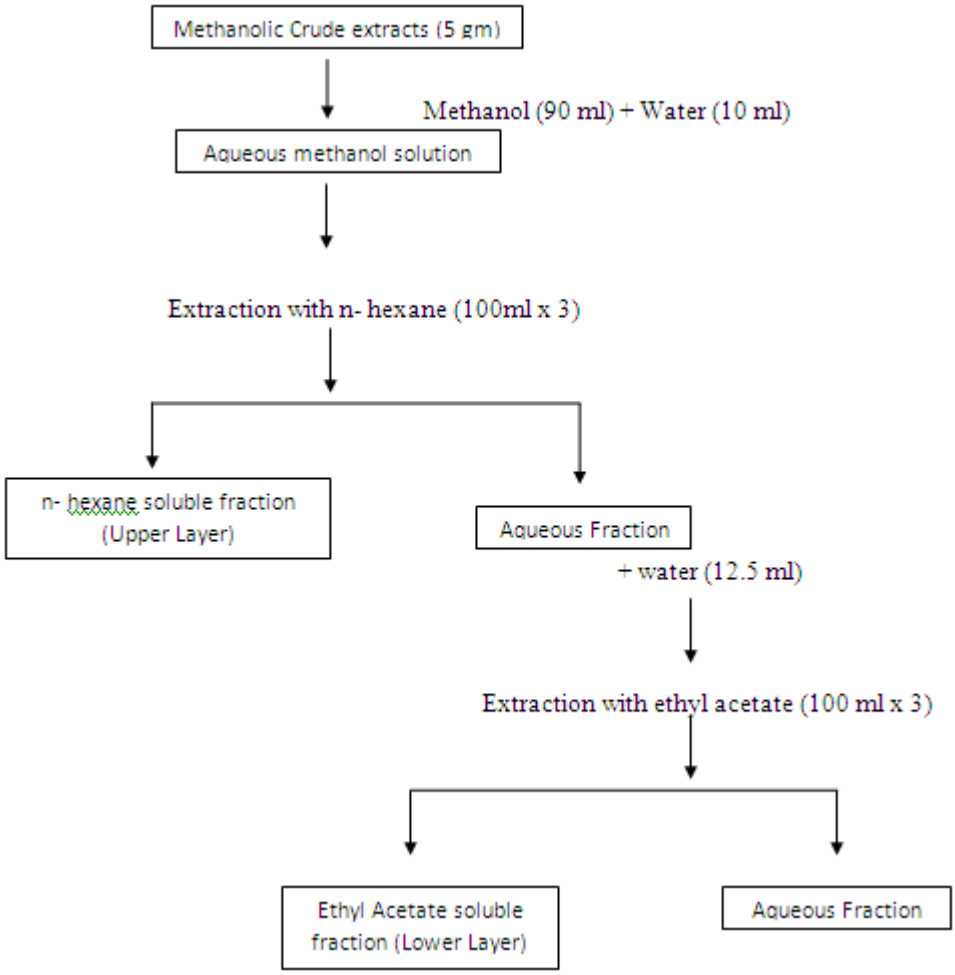



**Figure I**
***Schematic representation of modified Kupchan Partitioning of methanolic extract of Derris trifoliata***

***Isolation and Identification of compounds***

The hexane- soluble and ethyl acetate soluble mixtures were fractionated using column chromatography (Fig II). 30 gm of crude was mixed with 60 gms of Silica gel (60-120 mesh) to make the adhesive mixture. A column of diameter 2.4 cm and column bed height of 20 cm was packed with the adhesive mixture mixed with hexane. The column was eluted with increasing solvent polarity from hexane to ethyl acetate

**Figure II**
***Experimental set-up showing the isolation of Derris trifoliata leaf extracts using Column Chromatography***

### *I. By Thin Layer Chromatography*

For extraction, isolation and identification of active ingredients such as alkaloids, phenolics, terpenoids etc. solvent extraction procedure of Harborn[9] was adopted. Extracts obtained as above were concentrated to 1ml and 20µl loaded on TLC plates (Silica gel G 0.2 ml) and developed by the following solvents: Hexane: Ethyl acetate (1:1). The spot was observed on the TLC plates observed on the TLC plates and Rf value was calculated by using the following formula. RF = distance traveled by center of component /distance traveled by solvent front Rf value signifies the retention factor i.e., more the molecular weight the more will be the distance traveled by the isolates.

### *II. Detection of compounds by HPLTC*

Following extraction, Derris trifoliata standard and extracted samples were processed on the automated HPTLC system (CAMAG, Muttenz, Switzerland) according to the instructions of the manufacturer. The sample was sonicated to ensure complete solubility of extract, as well as filtration or centrifugation to remove undissolved excipients[10-13]. Ready-to-use silica coated plates (Manufacturer: E.MERCK KGaA) were activated by blowing hot air for 5-10 min and placed in the automatic sample applicator. Precoated TLC Silica Gel 60 F 254 (Merck TLC Plates) plates were used as the stationary phase. Plates were developed with methanol and dried in oven at 120°C for 20 minutes in order to remove contamination[10-20].The HPTLC was programmed to automatically spray 5-10 µl of each sample in band form using specialized Hamilton syringe on one-side of the TLC plate in individual tracks. The TLC plate was developed in Hexane : Ethyl acetate : Formic acid : Acetic acid (ratio 60 : 40 : 10 : 10) (v/v) solvent system[21,22]. The plate was developed in the automated developing chamber (CAMAG) until the solvent front reached the maximum distance (80 mm distance in a typical 20 x 10 cm plate).The developed plate was dried with a plate drier and subjected to UV analysis (wavelength: 200-600 nm) in the dedicated UV detector. All tracks on the plate were scanned at user-defined wavelength (254nm, 366 nm and visible light) and individual Rf values of peaks were obtained.

# RESULTS

### *Chemical Characterization of Leaf extract of Derris trifoliata*

Semi quantitative estimation and identification of active principles of the crude leaf extracts of Derris trifoliata were performed by the TLC method (Table I). In the present study TLC separation of methanolic extract of the plant material present three different compounds as revealed by fluorescents spots when visualized under UV light (Fig III).Three different compounds were isolated. The compound 1 Yellow Gel was collected from the fractions 21-26. Compound 2 White powder was collected from the fractions 42-50 and the compound 3 Brown powder was collected from the fractions 41-51 (Table I). The $R_f$ value of the three different spots were determined (Table II).

**Table I**

***Compounds isolated from Derris trifoliata using Column Chromatography***

| S.No | Number of Fractions | % of Solvent | Volume of Solvent (ml) | TLC Spot | Mobile phase |
|---|---|---|---|---|---|
| 1 | 1-20 | 100% Hexane | 500 | | H:Etoac(7:3) |
| 2 | 21-26 | 97%Hex: 3% Etoac | 200 | Yellow gel liquid | H:Etoac(7:3) |
| 3 | 27-36 | 95%Hex :5% Etoac | 250 | | H:Etoac(7:3) |
| 4 | 36-41 | 90%Hex:10% Etoac | 300 | | H:Etoac(7:3) |
| 5 | 41-51 | 87%Hex:13% Etoac | 500 | Brown powder | H:Etoac(6:4) |
| 5 | 42-50 | 85%Hex:15% Etoac | 500 | White powder | H:Etoac(6:4) |
| 6 | 51-56 | 80%Hex :20%Etoac | 250 | | |
| 7 | 57-61 | 70%Hex:30% Etoac | 250 | | |
| 8 | 62-70 | 100% Etoac | 100 | | |

**Figure III**

***Photograph of TLC plate under UV light, solvent – hexane: ethyl acetate (1:1)***

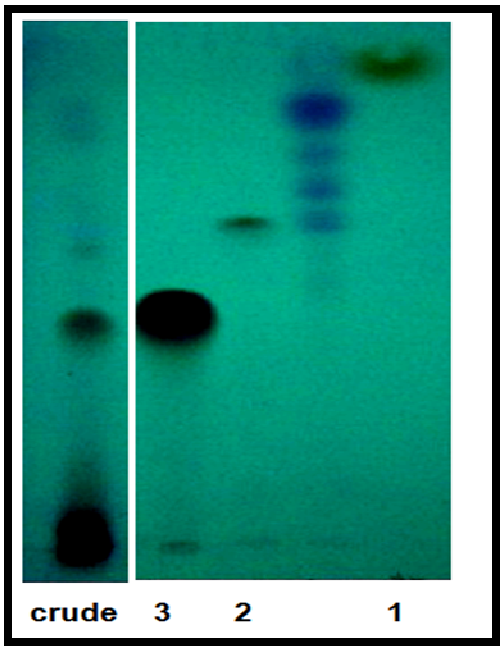


**Table II**
***Thin Layer Chromatography of leaf extracts and their $R_f$ values.***

| **Solvent** | **Hexane: Ethyl acetate (1:1)** | |
|---|---|---|
| **Material** | **Methanolic extract of Derris trifoliata** | |
| Compound | $R_f$ value | Iodine Vapour |
| 1 | 2.296 | Dark Green |
| 2 | 1.566 | Brown |
| 3 | 1.185 | Dark Brown |

To further characterize the components of Derris trifoliata, the three different compounds were subjected to HPTLC analysis in different solvent. Based on preliminary experiments, it was found that the metabolites exhibited different Rf values (Table III, IV & V). HPTLC based separation of Derris trifoliata extracts was done using Hexane : Ethyl acetate : Formic acid : Acetic acid (ratio 60 : 40 : 10 : 10) solvent system. Fig. IV, V and VI show the spectral scanning curve of the metabolites of Derris trifoliata. The plates were heated to remove the mobile phase the zones with the fluorescence were viewed under Duterium ($D_2$) and tungsten (W) lamps. The chromatogram was scanned using CAMAG TLC Scanner at 298 nm[9-12,20].

**Table III**
***$R_f$ values of individual peaks of compound 1 of Derris trifoliate***

| Peak | Start Rf | Start Height | Max Rf | Max Ht | Max % | End Rf | End Height | Area | Area % | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 0.1 | 0.06 | 15.1 | 0.37 | 0.07 | 0.5 | 128.1 | 0.1 | unknown* |
| 2 | 0.08 | 0.5 | 0.1 | 6.7 | 0.16 | 0.11 | 0.1 | 91.6 | 0.07 | unknown* |
| 3 | 0.15 | 0.2 | 0.17 | 99.8 | 2.43 | 0.2 | 38.2 | 2143.5 | 1.61 | unknown* |
| 4 | 0.2 | 38.4 | 0.24 | 224.4 | 5.47 | 0.27 | 163.3 | 7483.7 | 5.63 | unknown* |
| 5 | 0.27 | 163.8 | 0.28 | 172.2 | 4.19 | 0.28 | 172 | 2033.3 | 1.53 | unknown* |
| 6 | 0.28 | 172.4 | 0.3 | 219.5 | 5.35 | 0.32 | 175.4 | 5303.9 | 3.99 | unknown* |
| 7 | 0.32 | 176.6 | 0.34 | 308.3 | 7.51 | 0.35 | 259.9 | 6307.8 | 4.74 | unknown* |
| 8 | 0.35 | 261.7 | 0.38 | 328.2 | 7.99 | 0.4 | 241.3 | 10512.1 | 7.9 | unknown* |
| 9 | 0.4 | 243.5 | 0.43 | 556.3 | 13.55 | 0.48 | 142.5 | 22107.5 | 16.62 | unknown* |
| 10 | 0.48 | 143.3 | 0.51 | 385.9 | 9.4 | 0.54 | 198.6 | 11779.9 | 8.86 | unknown* |
| 11 | 0.54 | 199.3 | 0.56 | 243.1 | 5.92 | 0.59 | 96.3 | 7828.4 | 5.89 | unknown* |
| 12 | 0.59 | 96.6 | 0.63 | 491.7 | 11.98 | 0.7 | 53.1 | 19234.5 | 14.46 | unknown* |
| 13 | 0.7 | 53.5 | 0.78 | 308.1 | 7.5 | 0.8 | 223.1 | 14216.2 | 10.69 | unknown* |
| 14 | 0.8 | 224.1 | 0.81 | 263.2 | 6.41 | 0.87 | 135.8 | 10082.4 | 7.58 | unknown* |
| 15 | 0.87 | 136 | 0.89 | 186.8 | 4.55 | 0.9 | 179 | 4485.9 | 3.37 | unknown* |
| 16 | 0.9 | 179.1 | 0.93 | 296.2 | 7.21 | 0.98 | 0.6 | 9263.4 | 6.96 | unknown* |

**Table IV**
***$R_f$ values of individual peaks of compound 2 of Derris trifoliate***

| Peak | Start Rf | Start Height | Max Rf | Max Ht | Max % | End Rf | End Height | Area | Area% | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 0.7 | 0.06 | 17.2 | 0.39 | 0.07 | 1.1 | 152.3 | 0.1 | unknown* |
| 2 | 0.08 | 1.6 | 0.1 | 7.8 | 0.18 | 0.12 | 0 | 109.9 | 0.07 | unknown* |
| 3 | 0.14 | 0.4 | 0.17 | 110.8 | 2.5 | 0.19 | 48.7 | 2441.9 | 1.58 | unknown* |
| 4 | 0.19 | 48.9 | 0.23 | 243.4 | 5.49 | 0.26 | 156.4 | 8224.2 | 5.33 | unknown* |
| 5 | 0.26 | 156.5 | 0.3 | 255 | 5.76 | 0.31 | 222.4 | 7531.8 | 4.88 | unknown* |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 6 | 0.31 | 224.5 | 0.33 | 338.4 | 7.64 | 0.35 | 273.6 | 7384.2 | 4.78 | unknown* |
| 7 | 0.35 | 274.3 | 0.37 | 354.9 | 8.01 | 0.39 | 300.5 | 10023.8 | 6.49 | unknown* |
| 8 | 0.39 | 300.5 | 0.42 | 534.8 | 12.07 | 0.47 | 170.8 | 23037.2 | 14.92 | unknown* |
| 9 | 0.47 | 171.8 | 0.5 | 423.1 | 9.55 | 0.52 | 238.6 | 13030.9 | 8.44 | unknown* |
| 10 | 0.52 | 239.9 | 0.54 | 298.3 | 6.73 | 0.58 | 137.7 | 9633 | 6.24 | unknown* |
| 11 | 0.58 | 138.1 | 0.61 | 527.9 | 11.92 | 0.68 | 68.8 | 21719.7 | 14.07 | unknown* |
| 12 | 0.68 | 69.2 | 0.76 | 367.8 | 8.3 | 0.79 | 261.9 | 18492.7 | 11.98 | unknown* |
| 13 | 0.79 | 262 | 0.8 | 330.7 | 7.47 | 0.86 | 170.9 | 13756.3 | 8.91 | unknown* |
| 14 | 0.86 | 171.1 | 0.89 | 240.3 | 5.42 | 0.9 | 226.1 | 6788.9 | 4.4 | unknown* |
| 15 | 0.9 | 226.1 | 0.93 | 378.9 | 8.55 | 0.98 | 0.3 | 12028.4 | 7.79 | unknown* |

## Table V

### *$R_f$ values of individual peaks of compound 3 of Derris trifoliate*

| Peak | Start Rf | Start Height | Max Rf | Max Ht | Max % | End Rf | End Height | Area | Area% | Assigned substance |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.05 | 0 | 0.06 | 13.9 | 0.29 | 0.07 | 0 | 109.2 | 0.06 | unknown* |
| 2 | 0.08 | 0 | 0.1 | 7.5 | 0.16 | 0.12 | 0.1 | 90.3 | 0.05 | unknown* |
| 3 | 0.14 | 0 | 0.17 | 115 | 2.39 | 0.19 | 57.1 | 2694.3 | 1.58 | unknown* |
| 4 | 0.19 | 57.9 | 0.23 | 251.7 | 5.24 | 0.26 | 142.6 | 8551 | 5.03 | unknown* |
| 5 | 0.26 | 142.7 | 0.3 | 256.8 | 5.34 | 0.31 | 235.4 | 7091.2 | 4.17 | unknown* |
| 6 | 0.31 | 236.5 | 0.33 | 322.2 | 6.7 | 0.35 | 298 | 7953.4 | 4.67 | unknown* |
| 7 | 0.35 | 298.3 | 0.37 | 361.7 | 7.52 | 0.38 | 355.4 | 8832.7 | 5.19 | unknown* |
| 8 | 0.38 | 355.8 | 0.41 | 498.9 | 10.38 | 0.47 | 216.3 | 26626 | 15.65 | unknown* |
| 9 | 0.47 | 216.8 | 0.5 | 434.3 | 9.03 | 0.53 | 282 | 14616.5 | 8.59 | unknown* |
| 10 | 0.53 | 282.6 | 0.55 | 313.4 | 6.52 | 0.58 | 167.1 | 10613.1 | 6.24 | unknown* |
| 11 | 0.58 | 169 | 0.62 | 535.4 | 11.14 | 0.68 | 163.2 | 24570.5 | 14.44 | unknown* |
| 12 | 0.68 | 103.7 | 0.71 | 212 | 4.41 | 0.71 | 210.6 | 3295 | 1.94 | unknown* |
| 13 | 0.71 | 210.9 | 0.77 | 406.9 | 8.46 | 0.79 | 292.4 | 18306.7 | 10.76 | unknown* |
| 14 | 0.79 | 294.5 | 0.81 | 375.1 | 7.8 | 0.86 | 207.8 | 15213.6 | 8.94 | unknown* |
| 15 | 0.86 | 207.8 | 0.89 | 282.1 | 5.87 | 0.9 | 266.3 | 7568 | 4.45 | unknown* |
| 16 | 0.9 | 266.4 | 0.92 | 420.7 | 8.75 | 0.98 | 0.4 | 14008.1 | 8.23 | unknown* |

**Figure IVA**

***and IV B show the spectral scanning of compound 1 of Derris trifoliata with absorbance (AU) plotted against wavelength.***

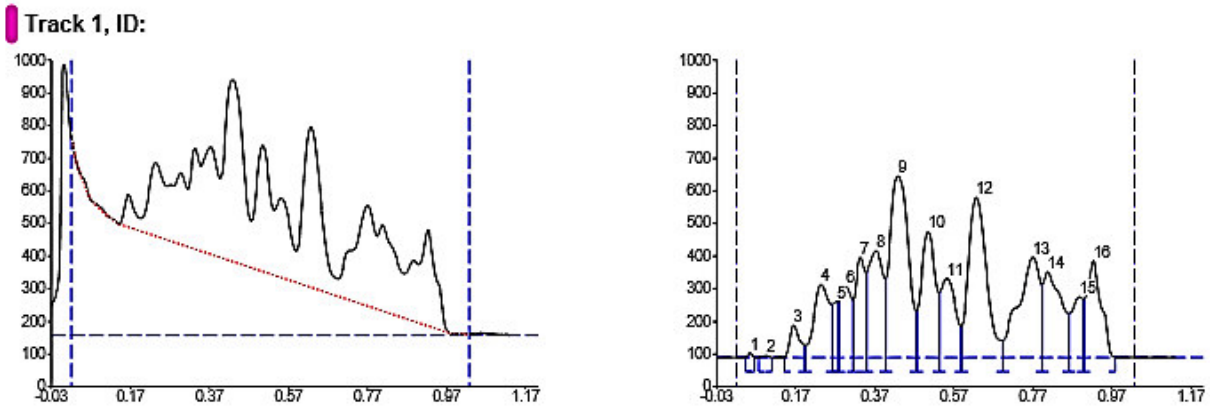



**Figure VA**

***and VB shows the spectral scanning of compound 2 of Derris trifoliata with absorbance (AU) plotted against wavelength.***

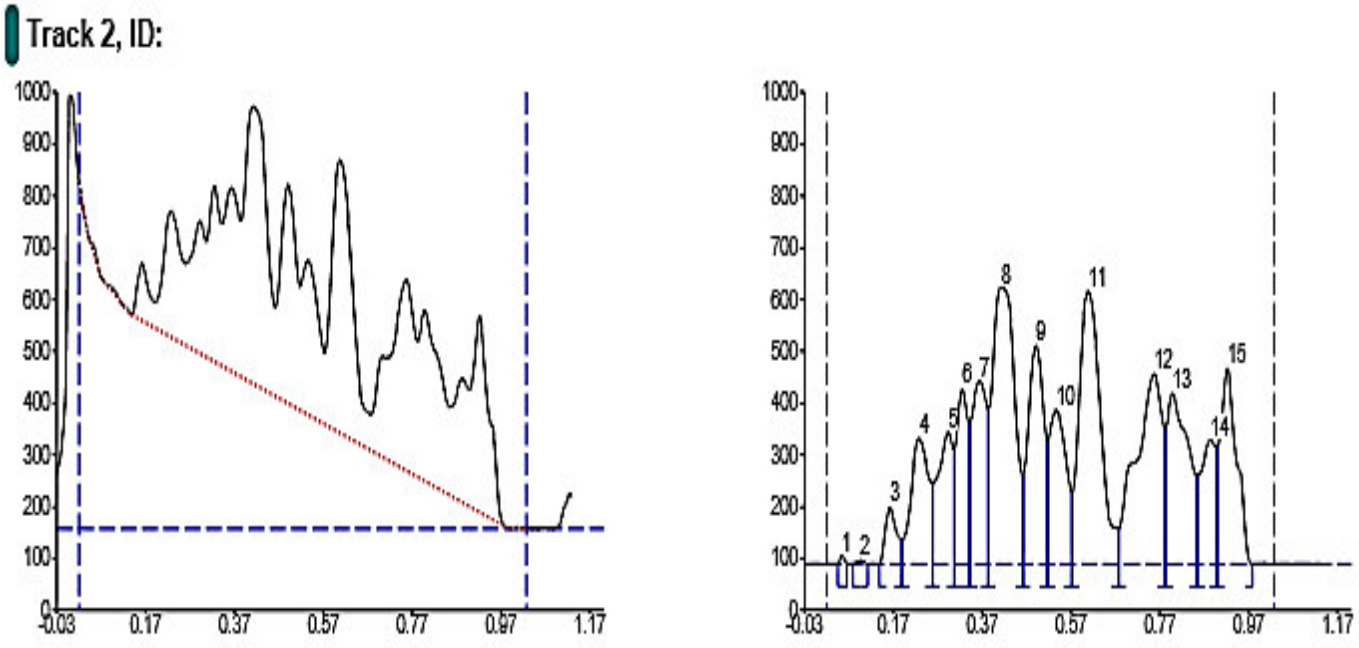


**Figure VI A**
***and VI B shows the spectral scanning of compound 3 of Derris trifoliata with absorbance (AU) plotted against wavelength***.

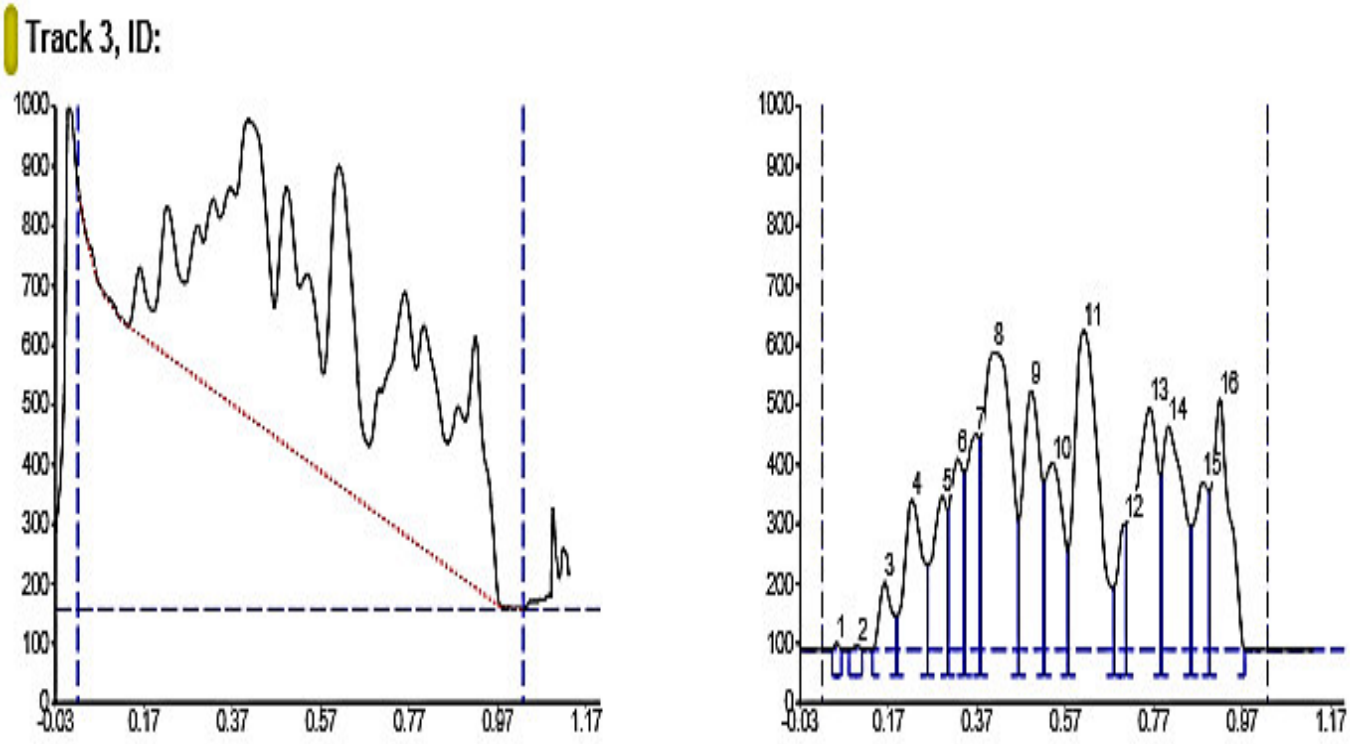
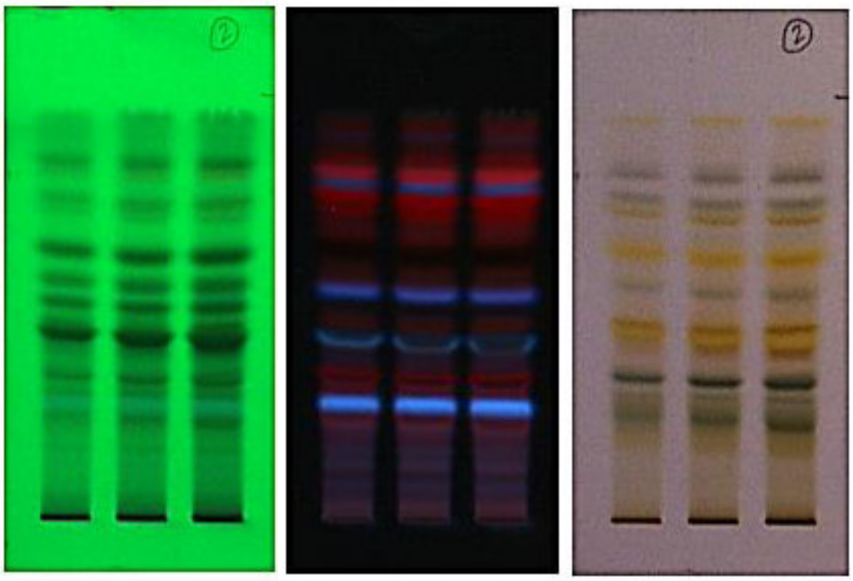



**Figure VII**
***HPTLC profile of Derris trifoliata extract (a) at 254 nm (b) at 254 nm (c) at visible light***



**(a)** **(b)** **(c)**

# DISCUSSION

### *Isolation of Compounds*

The plant leaves were extracted with methanol and then the phytochemical screening analysis was conducted. The methanolic leaf extracts were fractionated by column chromatography by using increasing polarity of Hexane to ethyl acetate solvent system.The thin layer chromatography of methanolic extract of Derris trifoliata leaves furnished three compounds with $R_f$ value 2.296, 1.566, 1.185 in hexane: ethyl acetate (1:1)

### *Characterization of Compounds*

Compound 1 was obtained as white crystalline solid. The TLC of the compound showed no fluorescence under UV light but showed a spot when put in iodine vapor. Compound 2 was obtained as grey powder. The TLC of the compound showed a single spot in iodine vapor. Compound 3 was obtained was a light yellow gel. The TLC of the last compound showed a spot in iodine vapor.For reliable psychiatric correlation, there needs to be well-standardized methods. Although ready-to-use cassettes are available, it has limited utility. Because it is relatively expensive and has a limited sensitivity threshold, and gives only qualitative and not absolute quantification. Most of the commercial kits clearly state that the test provides only a preliminary result and more specific alternative testing method should be used to confirm the immunoassay result[23]. This could be by either HPTLC or GC/MS or HPLC[24,25]. In routine TLC testing, the detection is only by a spray method and the Rf value is not accurately recorded[26]. However, UV based scanning after developing HPTLC plate not only provides opportunity for scanning at specific wavelengths, but could also be useful for quantitation.The phytochemical screening[28] revealed the presence of phytoconstituents such as alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids

# CONCLUSION

Medicinal plants used in the folk medicine may be an interesting and largely unexplored source for the development of potential new compounds[21]. But it is necessary to isolate the active principles and characterized their constituents for the beneficial of human being. It was our attempt to identify the new compounds in this plant that revealed three compounds. The purity of the compounds was determined for the effective utilization of the compounds in the production of novel pharmaceutical preparations.The plant kingdom offers a way of hope because of its enormous chemical diversity[29]. Several known anticancer drugs have been derived from medicinal plants and some of these include vincristine, vinblastine and taxol[30]. These compounds were isolated for the first time from this plant and the literature review revealed that the leaf extract of Derris trifoliata has much biological activities, including stimulant, antispasmodic, counter-irritant, rheumatism, chronic paralysis, and dysmenorrheal, laxative, carminative and anti-arthritis agent[6]. This attempt of phytochemical investigation from *Derris trifoliata*, further isolation and purification of other fractions of this plant is recommended which could yield some novel and bioactive compounds.

## REFERENCES


1. Ghani A, "Medicinal plants of Bangladesh with chemical constituents and uses", 2nd ed,: Asiatic society of Bangladesh, Dhaka, Ramna. p42, (2003)
2. B Sandhya, S Thomas, W Isabel, R Shenbagarathai, *Complementary and alternative medicines*, 3: 101- 114, (2006).
3. AJ Akindele, OO Adeyemi, *Fitoterapia*, 78: 25-28, (2007).
4. Peter K L Ng and N Sivasothi, "A Guide to the Mangroves of Singapore I: The Ecosystem and Plant Diversity", Singapore Science Centre, (p.108: description, habit, photo),(1999).
5. Tony Whitten and Jane Whitten (ed.), "Indonesian Heritage: Plants", Plants used as Medicine by Trond Schumacher, Editions Didier Millet, p. 69: uses, (1996).
6. Orwa C, Mutua A , Kindt R , Jamnadass R, Simons A, Agroforestree Database:a tree reference and selection guide version 4.0, (2009).
7. Kupchan SM, Tsou G, Tumor inhibitors. A new antileukemic simaroubolide from Brucea antidysenterica. J. Org. Chem.: 38: 178-179, (1973).
8. Wagenen BCV., Larsen R, Cardellina JH, Ran Dazzo D, Lidert ZC, Swithenbank C, Ulosantoin, a potent insecticide from the Sponge Ulosa ruetzleri. J. Org. Chem., 58: 335-337, (1993).
9. Harbornl, Phytochemical Methods, Chapman and Harborn, (1998).
10. Camag planar chromatography catalog; CAMAG Scientific Inc.: Wilmington, NC, ,http://www.camag.com. (Accessed on 01.04.2012), (2010/11)
11. Camag parameters for planar chromatography – useful hints, CAMAG, Muttenz,Switzerland, http://www.camag.com. (Accessed on 01.04.2012)
12. Koll, K., Reich, E. and Blatter, A, Validation of standardized high-performance thin-layer chromatographic methods for quality control and stability testing of herbals. J. AOAC Int. 86: 909-915, (2003).
13. Sherma. J, Review of HPTLC in drug analysis: 1996-2009. J. AOAC Int. 93: 754-764, (2010).
14. Jaenchen, D. E. and Reich. E, Planar chromatography: instrumentation. In "Encyclopedia of separation science". pp. 839-847, (2000)
15. Sherma. J, Chromatographic methods of analysis: thin layer chromatography. In "Encyclopedia of pharmaceutical technology". 3rd ed. pp. 538-550, (2007).
16. Sherma, J. and Fried, B, Handbook of Thin Layer Chromatography. 2nd ed. Marcel Dekker, Inc., New York, USA, (1996).
17. Sethi, P. D, High Performance Thin Layer Chromatography, Quantitative Analysis of Pharmaceutical Formulations. CBS Publishers, New Delhi, India, (1996).
18. Renger, B, Quantitative planar chromatography as a tool in pharmaceutical analysis. J. AOAC Int. 76: 7-13, (1993).
19. Renger. B, Contemporary thin layer chromatography in pharmaceutical quality control. J. AOAC Int.81: 333-339, (1998).
20. Patel, R. B., Patel, M. R. and Patel, B. G Experimental aspects and implementation of HPTLC. In "High performance thin layer chromatography (HPTLC)". pp. 41-54, (2011).
21. Harvey DJ, Paton WD. Use of trimethylsilyl and other homologous trialkylsilyl derivatives for the separation and characterization of mono and di-hydroxy cannabinoids by combined gas chromatography and mass spectrometry. J Chromatogr, 109 : 73-80, (1975).
22. Frederick DL, Green J, Fowler MW. Comparison of six cannabinoid metabolite assays. J Anal Toxicol, 9 : 116-20, (1985).
23. Altunkaya D, Clatworthy AJ, Smith RN, Start IJ. Urinary cannbinoid analysis: comparison of four immunoassays with gas chromatography-mass spectrometry. Forensic Sci Int, 50 : 15-22, (1991).
24. Weaver ML, Gan BK, Allen E, Baugh LD, Liao FY, Liu RH, et al. Correlations on radioimmunoassay, fluorescence

polarization immunoassay and enzyme immunoassay of cannabis metabolites with gas chromatography/mass spectrometry analysis of 11-nor-Δ9-tetrahydrocannabinol 9-carboxylic acid in urine specimens. Forensic Sci Int, 49 : 43-56, (1991).
25. Abraham TT, Lowe RH, Pirnay SO, Darwin WD, Huestis MA. Simultaneous GC-EI-MS determination of Δ9-tetrahydrocannabinol, 11-hydroxy-Δ9-tetrahydrocannabinol, and 11-nor-9-carboxy-Δ9-tetrahydrocannabinol in human urine following tandem enzyme-alkaline hydrolysis. J Anal Toxicol, 31 : 477-85, (2007).
26. Nikonova EV, Karaeva LD. Use of thin-layer chromatography for detection of 11-nor-9-carboxy-delta 9-tetrahydrocannabinol in urine. Sud Med Ekspert, 48 : 33-5, (2005).
27. Priya G , Chellaram C, Evaluation of antibacterial activity and phytochemical analysis of medicinal plant solanum trilobatum. Int J Pharm Bio Sci; 5(3): (P) 354 – 35,9 (2014)
28. Radulovic NS, Blagojevic PD, Stojanovic-Raclic ZZ, Stojanovic NM. Antimicrobial plant metabolites: structural diversity and mechanism of action. Curr Med Chem, 20(7): 932-952, (2013).
29. Rasoanaivo P, Ratsimamanga Urverg S. Biological evaluation of plants with reference to the Malagasy flora. Monograph prepared for the IFS-NAPRECA workshop on bioassay held in Antananarivo, Madagascar. Antananarivo: NAPRECA, p. 65-71; (1993).