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# Effect of Extraction Solvents on the Phytoconstituents of Different Parts of *Wedelia Trilobata* L. - A Tropical Perennial *Medicinal Herb*

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**Abstract**
The objective of the present study was to evaluate the effect of extraction solvents such as n-Hexane, Petroleum ether, Chloroform, Methanol and aqueous Methanol (Methanol: Water, 50:50 v/v) on phytochemicals of different parts like leaf, stem and flower of *Wedelia trilobata* using chromatographic studies. The chemical pattern of different extracts was compared using Thin Layer Chromatography (TLC). Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents. Chloroform extracts of leaf, stem and flower showed highest number of phytochemicals than Hydro-alcohol, Methanol, Petroleum ether and n-Hexane extracts. Due to the higher number of phytochemicals of extract, use of Chloroform for the preparation of herbal formulations can be considered after proper phytochemical characterization, identification of bioactive compounds and quality standard tests and it may lead to the judicious use of raw materials.

**Keywords:** *Wedelia trilobata, Medicinal herb*, Extraction solvent, TLC.

## Introduction
Plant-derived substances are always an area of interest for research due to their huge number of applications in traditional, folk and modern medicines, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. However, plants are currently in considerable significance as a large source of therapeutic phytochemicals having various medicinal properties.

The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive compounds from plant materials. Extraction (pharmaceutically) is the separation of medicinally active portions of plant tissues using selective solvents through standard procedures [2]. Solvents diffuse into the solid plant material and solubilize compounds with similar polarity during extraction [1]. The products obtained from plant parts using extraction procedures are relatively complex mixtures of metabolites. They are subjected to further processing procedures to obtain therapeutic phytochemicals of interest.

*Wedelia* is a genus of the family Asteraceae, comprising about 60 different species. *Wedelia trilobata* Linn. has long been used as traditional herbal medicine in South America, China, Japan, India and for the treatment of a variety of ailments[3]. Presence of secondary metabolites and phytoconstituents like terpenoids, flavonoids, polyacetylenes, steroids, eudesmanolide lactones, luteolin and kaurenoic acid has been reported in different parts of this plant [4-9]. Hepatoprotective, antibacterial, anti- hemorrhagic, antiepileptic activities were found exhibited by wedelolactone- a compound which was found to be present in *W.trilobata*[10-12]. Antifungal, anti-inflammatory, antioxidant, analgesic and anti-diabetic activities by different solvent extracts of different parts of *W. trilobata* were reported [9, 13, 14].

It is important to find solvents and extraction techniques to efficiently isolate and separate maximum amount of phytochemicals of interest from minimum amount of plant samples, thereby increasing the yield of raw materials. Aim of this investigation is to study the effect of extraction solvents like Hexane, petroleum ether, chloroform, methanol and hydro-alcohol on phytochemicals of leaf, stem, and flower of *Wedelia trilobata* and thereby compare the ability of these solvents to extract different phytochemicals of different polarity.

## Materials and Methods

### Sample collection

The plant sample *Wedelia trilobata* was collected from Kozhikode district, Kerala and authenticated by Dr. Satheesh George, Department of Botany, St. Joseph's College, Devagiri. Plant material was cleaned well in running water, dried in hot air oven at 40°C and leaf, stem and flower were separately ground to fine powder using electric blender. Powdered samples were kept away from sunlight in air tight containers at room temperature.

### Chemicals used

Methanol (HPLC grade), Chloroform, Petroleum ether and n-Hexane purchased from Merck and SIGMA-ALDRICH were used as solvents. Toluene and Ethyl acetate purchased from Merck were used in TLC studies.

### Extraction

Leaf, stem and flower samples were extracted in n-Hexane, Petroleum ether, Chloroform, Methanol and Hydro-alcohol (methanol : water=1:1) using reflux condenser. 4g of each sample was taken in a RB flask with 100mL of solvent and boiled for 3 hours. These extracts were filtered using Whatman No.1 filter paper and concentrated to 20mL on a water-bath. These extracts were kept in amber bottles and preserved in refrigerator.

### Thin Layer Chromatography (TLC) studies

Thin Layer Chromatography (TLC) profiling of different extracts of leaf, stem and flower of *Wedelia trilobata* was carried out on pre-coated silica gel plates (60 $F_{254}$ Merck) using toluene, ethyl acetate and methanol as mobile phase in the ratio 7:3:1. Different solvent extracts of same plant part were spotted on a single plate as thin bands. The developed plates were observed under visible spectrum, UV 254nm and 366nm and observations were recorded. $R_f$ values of each compounds visible as different bands were calculated using the formula –

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent front TLC plates}}$$

## Results and Discussion

Authentication of medicinal plants at chemical level is a crucial step for both research purposes and medicinal preparations. TLC fingerprints of extracts can be used as an identity of plants. The chemical pattern of different extracts was compared in this investigation using TLC profiling. Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents.

### Leaf

Maximum number of bands was observed for Chloroform extract (total 10 bands) and 2 unique bands of $R_f$ = 0.54 and 0.86 were also recorded. Least number of bands was recorded for Hydro-alcohol extract (total 5 bands). A total of 8 bands for methanolic extract and 7 for both Petroleum ether and n-Hexane extracts were recorded. The TLC data of leaf extracts is given in Fig.1 and Table-1.

### Flower

Best number of bands was observed for Chloroform extract (total 6 bands) and 2 unique bands of $R_f$ = 0.38 and 0.33 were also recorded. No band was recorded for Hydro-alcohol extract. A total of 4 bands for Petroleum ether extract, 3 bands for n-Hexane extract and 1 light band for methanolic extract were recorded. The TLC data of flower extracts is given in Fig.2 and Table-2.

### Stem

Greatest number of bands was observed for Chloroform extract (total 8 bands). Only a single light band was exhibited by Hydro-alcohol extract. A total of 7 bands for methanolic extract and 5 bands for both Petroleum ether and n-Hexane extracts were recorded. The TLC data of stem extracts is given in Fig.3 and Table-3.

Chloroform extracts exhibited the most number of bands among 5 different extracts, thus Chloroform exhibited high ability for the extraction of phytochemicals in *Wedelia trilobata* in TLC studies. Most number of bands was recorded in leaf extract and this indicates the relatively high presence of phytochemicals in the leaf among different parts of *W. trilobata.*

Chloroform was reported as a good solvent for the extraction of intermediately-polar constituents like terpenoids and flavonoids [15]. Chloroform leaf extract was reported to exhibit more number of bands than methanol and petroleum ether extracts of *Senna siamea* in TLC studies. The presence of saponins in chloroform leaf extract and anthraquinone in flower extract was also reported in the same study [16]. Concomitant with our study, qualitative phytochemical screening of *Wedelia biflora* leaf extract using various solvents showed that most of the biologically active phytochemicals are present in chloroform extract. The presence of alkaloids and tannins in the chloroform leaf extract was also identified in this investigation [17]. Contrasting results were also reported in a comparative study of flower extracts, in which presence of more phytochemicals was identified in methanolic flower extract than in chloroform extract of *Wedelia trilobata* [18].

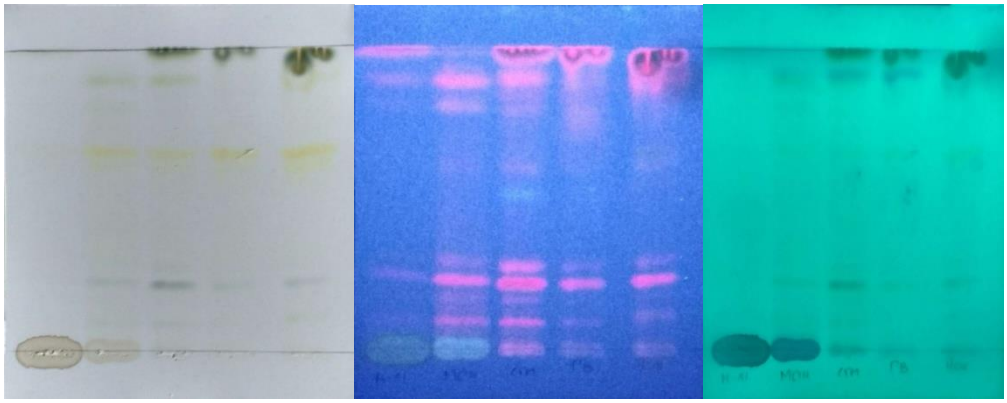

**Fig. 1:** developed TLC plates with leaf extracts at visible range, 366nm and 254nm respectively.

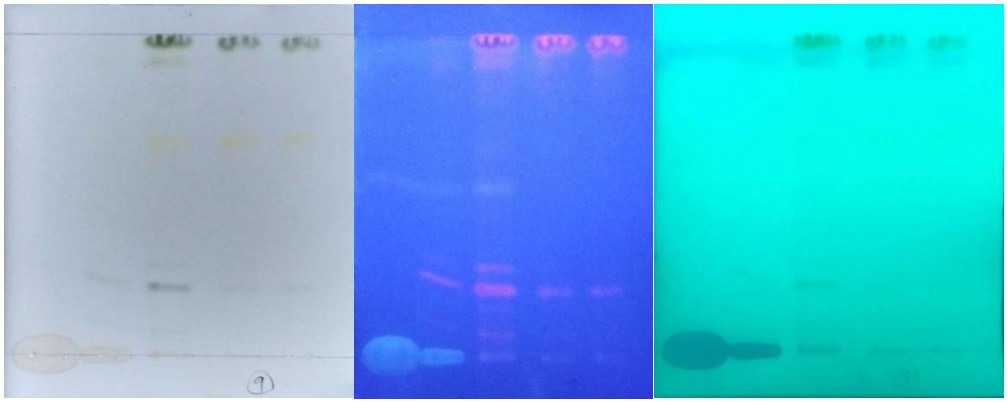

**Fig. 2:** developed TLC plates with flower extracts at visible range, 366nm and 254nm respectively.

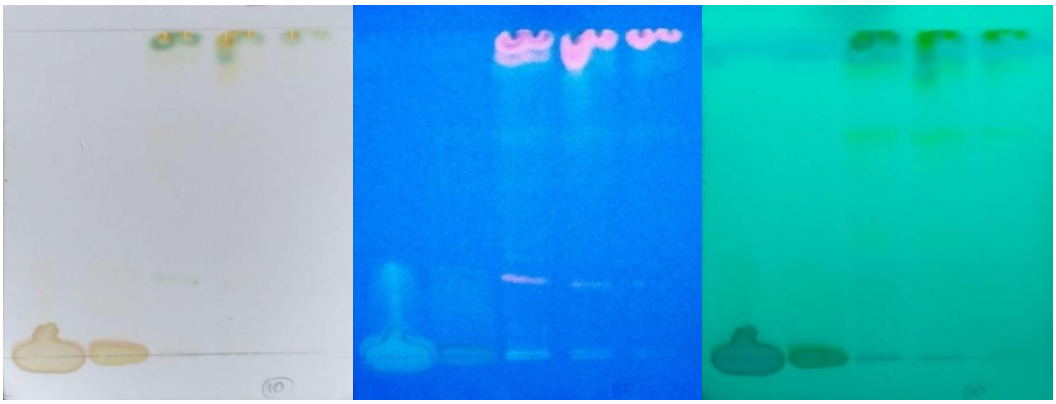

**Fig. 3:** developed TLC plates with stem extracts at visible range, 366nm and 254nm respectively.

**Table 1:** $R_f$ values of components in Leaf extracts.

| $R_f$ values - Visible range | | | | | 366nm | | | | | 254nm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** |
| 0.91* | 0.91 | 0.91 | _ | _ | 0.91 | 0.91 | 0.91 | 0.91 | 0.91 | _ | 0.91 | 0.91 | 0.91 | _ |
| _ | _ | _ | _ | _ | _ | _ | 0.86 | _ | _ | _ | _ | _ | _ | _ |
| _ | 0.83 | _ | _ | _ | 0.83* | 0.83 | 0.83 | 0.83 | _ | _ | _ | _ | _ | _ |
| 0.68 | 0.68 | 0.68 | 0.68 | 0.68 | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ |
| _ | _ | _ | _ | _ | _ | 0.63* | 0.63 | 0.63* | 0.63 | _ | 0.63 | 0.63 | 0.63 | 0.63 |
| _ | _ | _ | _ | _ | _ | _ | 0.54 | _ | _ | _ | _ | _ | _ | _ |
| _ | _ | _ | _ | _ | _ | 0.31 | 0.31 | 0.31 | 0.31 | _ | 0.31 | 0.31 | _ | _ |
| _ | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | _ | 0.23 | 0.23 | 0.23 | 0.23 |
| _ | _ | _ | _ | _ | _ | 0.18 | 0.18 | _ | 0.18 | _ | _ | _ | _ | _ |
| _ | 0.09 | 0.09 | _ | _ | 0.09* | 0.09 | 0.09 | 0.09 | 0.09 | _ | 0.09 | 0.09 | | 0.09 |

**(1-** Hydro-alcohol**, 2-** Methanol**, 3-** Chloroform**, 4-** Petroleum ether**, 5-** n-Hexane**)**

**Table 2:** $R_f$ values of components in Flower extracts.

| $R_f$ values - Visible range | | | | | 366nm | | | | | 254nm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** |
| _ | _ | 0.94 | 0.94 | 0.94 | _ | _ | 0.94 | _ | _ | _ | _ | 0.94 | 0.94 | 0.94* |
| _ | _ | 0.7 | 0.7 | 0.7* | _ | _ | 0.7 | 0.7 | 0.7 | _ | _ | 0.7 | 0.7 | 0.7 |
| _ | _ | _ | _ | _ | _ | _ | 0.56 | 0.56 | _ | _ | _ | _ | _ | _ |
| _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | _ | 0.38 | _ | _ |
| _ | _ | _ | _ | _ | _ | _ | 0.33 | _ | _ | _ | _ | _ | _ | _ |
| _ | _ | 0.25 | _ | _ | _ | 0.25* | 0.25 | 0.25 | 0.25 | _ | _ | 0.25 | _ | _ |

**(1-** Hydro-alcohol**, 2-** Methanol**, 3-** Chloroform**, 4-** Petroleum ether**, 5-** n-Hexane**)**

**Table 3:** $R_f$ values of components in Stem extracts.

| $R_f$ values - Visible range | | | | | 366nm | | | | | 254nm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** | **1** | **2** | **3** | **4** | **5** |
| _ | 0.94* | 0.94 | _ | _ | _ | 0.94 | 0.94 | 0.94 | 0.94 | _ | 0.94* | 0.94 | 0.94 | 0.94 |
| _ | _ | _ | _ | _ | _ | _ | 0.88 | 0.88 | 0.88 | _ | _ | _ | _ | _ |
| _ | 0.69* | 0.69 | 0.69 | 0.69 | _ | _ | 0.69 | _ | _ | _ | _ | 0.69 | 0.69 | 0.69 |
| _ | _ | _ | _ | _ | 0.56* | 0.56 | 0.56 | _ | _ | _ | _ | _ | _ | _ |

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| _ | _ | 0.3 | _ | _ | _ | 0.3 | 0.3 | _ | _ | _ | _ | _ | _ | _ |
| _ | 0.23 | 0.23 | 0.23* | 0.23* | _ | 0.23 | 0.23 | 0.23 | 0.23 | _ | 0.23 | 0.23 | _ | _ |
| _ | _ | _ | _ | _ | _ | 0.16* | 0.16 | _ | _ | _ | _ | _ | _ | _ |
| _ | _ | 0.08 | _ | _ | _ | 0.08 | 0.08 | 0.08* | 0.08* | _ | _ | 0.08 | _ | _ |

***low quantity**

**(1-** Hydro-alcohol**, 2-** Methanol**, 3-** Chloroform**, 4-** Petroleum ether**, 5-** n-Hexane**)**

## Conclusion

It is very important to obtain reliable chromatographic fingerprints of herbal medicines that represent pharmacologically active and chemically characteristic components in them, as they are composed of many constituents and are therefore very capable of variation.

TLC fingerprints of different extracts of leaf, stem and flower of *Wedelia trilobata* was obtained in this study. Different $R_f$ value of various phytochemicals provide valuable clue about their polarity and selection of solvents for the separation of these phytochemicals. The results of phytochemical analysis showed that the Chloroform extracts of leaf, stem and flower showed highest number of phytochemicals than Hydro-alcohol, Methanol, Petroleum ether and n-Hexane extracts. Due to the higher number of phytochemicals of extract, use of Chloroform for the preparation of herbal formulations can be considered after proper phytochemical characterization, identification of bioactive compounds and quality standard tests and it may lead to the judicious use of raw materials. Possibility of reducing the quantity of raw drugs can be explored if raw drugs are being extracted with chloroform instead of water.

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