

## Research Article

**Phytochemical Investigation of *Wedelia chinensis* (Osbeck.) Merrill****H. Rehana Banu<sup>1\*</sup> and N. Nagarajan<sup>2</sup>**<sup>1</sup>Department of Botany, Nirmala College for Women, Coimbatore, Tamilnadu, India.<sup>2</sup>Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India.**ABSTRACT**

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Hence in the present study an important medicinal herb *Wedelia chinensis* (Osbeck) Merrill was investigated for qualitative and quantitative phytochemical screening using standard methods. Preliminary phytochemical screening of various extracts of the leaves revealed the presence of various classes of compounds such as alkaloids, flavonoids, tannins and phenols. The results suggest that the phytochemical property of the plant for curing various ailments leads to the isolation of new and novel compounds.

**Keywords:** Traditional medicine, novel research, compounds and ailments.

**INTRODUCTION**

Plants contain hundreds or thousands of metabolites. Medicinal and aromatic plants are gifts of nature and are being used against various infections and diseases in the world since past history<sup>1</sup>. World Health Organization (WHO) has defined medicinal plants as plants that contain properties or compounds that can be used for therapeutic purposes or those that synthesize metabolites to produce useful drugs<sup>2</sup>. Among the estimated 2,50,000 to 5,00,000 plant species, only a small percentage has been investigated phytochemically. Plant kingdom represents an extraordinary reservoir of novel molecules. Plant derived products have been used for medicinal purposes for centuries<sup>3</sup> and plants have been an important source of medicine for thousands of years<sup>4</sup>. Medicinal plants have been an important resource of human health care from prehistoric times to the present day. Its not only important for pharmacological research and drug development, not only as plant constituents used directly as therapeutic agents, but also as starting materials for the synthesis of drugs or as models for pharmacologically active compounds<sup>5</sup>. India is a varietal emporium of medicinal and aromatic plants. It is one of the richest countries in the world with regard's to genetic resources of medicinal plants. It exhibits a wide range of topography and climate, which has a bearing on its vegetation and floristic composition<sup>6</sup>. It is one of the 12 mega biodiversity centers having 45,000 plant species and has a rich floral diversity<sup>7</sup>. All

plant parts synthesize many chemicals by themselves, to perform their physiological activities. The medicinal value of these secondary metabolites is due to the presence of chemical substances (phytochemicals) that produce a definite physiological action on the human body. Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, etc<sup>8</sup> i.e. any part of the plant may contain active components. The most important of these substances include alkaloids, glycosides, steroids, flavonoids, fatty oils, resins, mucilages, tannins, gums, phosphorus and calcium<sup>9,10</sup>.

The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bio-activities<sup>11</sup>. Hence, the present investigation was carried out with the aim to study the phytochemical constituents in the leaves of an important medicinal herb, *Wedelia chinensis* (Osbeck) Merrill.

**MATERIALS AND METHODS****Plant Material**

*Wedelia chinensis* (Osbeck) Merrill leaves (Fig. 1) were collected from Attakatti Hills, Western Ghats, India. The plant material was taxonomically identified by Dr. V. Balasubramanian, Associate Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India and was deposited at the college herbarium for future reference. The leaves were washed, shade dried and is made

powder mechanically and the fine powder was used for extraction procedure and phytochemical evaluation.

### Preparation of Extracts

For extraction about 50g of the shade dried and powdered leaf material was taken. The powdered material was transferred into 250 ml quick fit flask and extracted in the soxhlet extractor for 48 hours<sup>12,13</sup> using of organic solvents namely petroleum ether, chloroform, ethyl acetate and methanol separately according to the increasing polarity of the solvents. The extracts were filtered over Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass<sup>14</sup> for further studies.

### Qualitative Phytochemical screening

The extracts of the dry powdered leaves of *Wedelia chinensis* were analyzed for the presence of various phytochemical constituents. All preliminary phytochemical study was carried out using the following methodologies of<sup>15,16,17</sup>.

#### Test for Alkaloids

##### Dragendroff's test

8g of bismuth nitrate was dissolved in 20 ml of nitric acid and 2.72 g of potassium iodide in 50 ml of distilled water. They were mixed and allowed to stand when potassium nitrate crystals out. The supernatant was decanted off and made up to 100 ml with distilled water.

To 0.5 ml of alcoholic extract of *Wedelia chinensis* was added 2 ml of hydrochloric acid. To this acidic medium, 1 ml of reagent was added. An orange red precipitate produced immediately indicates the presence of alkaloids.

##### Wagner's test (Iodine-potassium-iodide solution)

1 g of iodine and 2 g of potassium iodide solution was diluted to 100 ml. 10 ml of alcoholic extract of *Wedelia chinensis* was acidified by adding 1.5% v/v of Hydrochloric acid and a few drops of Wagner's reagent. Formation of yellow or brown precipitate confirmed the presence of alkaloids.

##### Meyer's reagent (potassium mercuric iodide)

1.36 gm of mercuric chloride was dissolved in 60 ml of distilled water and 5 gm of potassium iodide was dissolved in 10 ml of water. These two solutions were mixed and diluted to 100 ml with distilled water. To 1 ml of the extract, a few drops of reagent were added. Formation

of white or pale precipitate showed the presence of alkaloids.

#### Test for Flavonoids

In test tube containing 0.5 ml of extract, 5 to 10 drops diluted hydrochloric acid and small piece of Zinc or magnesium were added and the solution was boiled for few minutes. The appearance of reddish pink or dirty brown colour indicates the presence of flavonoids.

#### Test for Saponins

In a test tube containing about 5 ml of the extract, few drops of sodium bicarbonate was added. The mixture was shaken vigorously and kept for 3 minutes. A honeycomb like froth was formed and it showed the presence of saponins.

#### Test for Phenols

##### i) Ferric chloride test

To 1 ml of the extract 3 ml of distilled water followed by few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicates the presence of phenols.

##### ii) Lead acetate test

1 ml of the extract was diluted to 5 ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate formation indicates the presence of phenols.

##### iii) Liebermann's test

A small quantity of extract was dissolved in 0.5 ml of 20% sulphuric acid solution followed by the addition of a few drops of aqueous sodium nitrate solution. A red colour was obtained on dilution and it turned blue when made alkaline with aqueous sodium hydroxide solution which confirmed the presence of phenols.

#### Test for Tannins

##### i) Lead acetate test

In a test tube containing about 5 ml of the extract, a few drops of 1 % solution of lead acetate was added. A yellow or red precipitate indicates the presence of tannins.

##### ii) FeCl<sub>3</sub> test

About 2.5g of the plant extract was dissolved in 5 ml of distilled water and it was filtered. Ferric chloride reagent was added to this filtrate. Development of blue black, green, blue green precipitate was observed that revealed the presence of tannins.

**Test for Glycosides**

A small amount of extract was dissolved in 1 ml of water and aqueous sodium hydroxide solution was added. Formation of yellow colour indicates the presence of glycosides.

**Test for Steroids****Liebermann-Burchard's test**

To 1 ml of extract in chloroform, 1 ml of concentrated sulphuric was added followed by 2 ml of acetic anhydride solution. A greenish colour developed and it turned blue. It indicates the presence of steroids.

**Salkowski reaction**

To 2.0 ml of extract, 1.0 ml of concentrated sulphuric acid was added carefully along the sides of the test tube. A red colour produced in the chloroform layer shows the presence of steroids.

**Test for Cardiac glycosides****Keller killiani test**

100 mg of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. Then, 1ml of concentrated sulphuric acid was added. A brown ring obtained at the interface indicated the presence of a de-oxysugar, a characteristic of cardenolides.

**Test for Anthraquinones****Borntrager's test**

0.5 ml extract was taken into a dry test tube and 5 ml of chloroform was added and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100% ammonia solution. Development of a pink violet or red colour in the ammoniacal layer (lower layer confirmed the presence of free anthraquinones).

**Test for Resins**

To 2.0 ml of extract 5 ml of acetic anhydride was added, dissolved by gently heating, cooling and then 0.5 ml of sulphuric acid was added. Bright purple colour indicates the presence of resins.

**Quantification****Determination of total phenols**

Total phenolics were quantified and expressed as gallic acid equivalents according to a method proposed by <sup>18</sup>. About 3.9ml of distilled water and 0.5ml of Folin-ciocalteau reagent were added to 0.1ml of methanolic extract of *Wedelia chinensis* in a tube and incubated at room temperature for 3 min after which 2 ml of 20% sodium carbonate was added to this and kept at boiling water bath for 1 min. Phenols

react with phosphomolybdic acid in the Folin-Ciocalteau reagent in alkaline medium and produce a blue coloured complex (molybdenum blue) that can be estimated colorimetrically at 650 nm.

**Determination total flavonoids**

Total flavonoid content was measured by the aluminium chloride colorimetric assay <sup>19</sup>. An aliquot (1 ml) of extract and standard solution of catechin (100 mg/ml) was added to 10 ml volumetric flask containing 4 ml of distilled water. To this 0.3 ml of 5 % NaNO<sub>2</sub> were added. After 5 min, 0.3 ml 10 % AlCl<sub>3</sub> was added. Then after 1 min, 2ml of 1 M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

**Determination of total alkaloids**

Determination of alkaloid was done as described <sup>20,21,22</sup>. 5 g of plant sample was weighed into a 250 ml beaker and dispersed into 200 ml of 10% acetic acid solution in ethanol. The filtrate was then evaporated to one quarter of its original volume on hot plate. Concentrated ammonium hydroxide was added drop wise in order to precipitate the extract. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

**Determination of total tannins**

Tannin content was measured by Folin-Denis method <sup>23</sup>. 50 µl of extract was made up to 7.5 ml by adding double distilled water. Then 0.5 ml of Folin Denis reagent and 1 ml of Na<sub>2</sub>CO<sub>3</sub> were mixed with it. Again volume was made up to 10 ml by double distilled water. Absorption was recorded at 700 nm. The vanillin reagent will react with any phenol that has an unsubstituted resorcinol or phloroglucinol nucleus and forms a coloured substitution product which is measured at 700 nm.

**RESULTS AND DISCUSSION****Preliminary phytochemical studies**

Phytoconstituents are the natural bioactive compounds found in plants. These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions. Phytochemicals are basically

divided into two groups, i.e. primary and secondary constituents; according to their functions in plant metabolism. Primary constituents comprise common sugars, amino acid, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, saponins, phenolic compounds, flavonoids, tannins, etc. These natural compounds paved the foundation for modern prescription drugs as we know today<sup>24</sup>. Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. These compounds are involved in allelopathy, dealing with the interactions between two plants, which process can change depending upon variations in the phytochemicals produced under particular environmental conditions<sup>25</sup>.

Phytochemical screening of plant products or extracts aimed to evaluate the antibacterial properties which may be of immense importance in the discovery of new therapeutic agents, especially at this time when the scientific community is preoccupied with searching for alternative treatment to combat the increasing threat of drug resistant microorganisms. The various organic solvent extracts of the leaves of *W. chinensis* were subjected to preliminary phytochemical tests. The results are depicted in Table 1. The results revealed the presence of alkaloids, flavonoids, phenols, tannins and saponins in the methanol, ethyl acetate and chloroform extracts. The petroleum ether extracts showed the absence of all the phytochemical constituents that were tested. These are the phytochemicals which are essential for the antioxidant property either by scavenging free radicals or by preventing their formation<sup>26</sup>.

The detected phytochemical components such as alkaloids, flavonoids, phenols and tannins were found to possess many biological activities. For instance: According to<sup>27</sup> and<sup>28</sup>, alkaloids are formed as metabolic by-products and have been reported to be responsible for the antibacterial activity. According to Lewis and Elvin-Lewis<sup>29</sup>, alkaloids functions with the aid of their defense mechanism act as phytoprotective agent against invading microorganism. Alkaloids have anti-inflammatory effects<sup>30,31,32</sup>, hypoglycemic activities<sup>33</sup>. A report by<sup>34</sup> indicates that

naturally occurring alkaloids and their synthetic derivatives have analgesic, antispasmodic and bactericidal activities.

Phenolic compounds are a large, heterogeneous group of secondary plant metabolites that are widespread in the plant kingdom. Plant phenolics are characterized as aromatic compounds, which possess one or more acidic hydroxyl group attached to phenyl ring. All the phenolic classes have received considerable attention because of their physiological functions, including free radical scavenging, antioxidants<sup>35</sup> and antimicrobial activity<sup>36</sup>. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metal chelating potential<sup>37</sup>.

Flavonoids, (a large group of naturally occurring plant phenolic compounds including flavones, flavonols, isoflavones, flavonones and chalcones) also known as nature's tender drugs possess numerous biological / pharmacological activities. Recent reports of antiviral, antifungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective and cytotoxic activities of flavonoids have generated interest in studies of flavonoid containing plants<sup>38,39,40,41,42,43</sup>.

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them<sup>44</sup>. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins<sup>45</sup>. Tannins are reported to have various physiological effects like anti-irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects.

#### Quantitative phytochemical screening

The results of the phytochemicals like alkaloids, flavonoids, phenols and tannins of methanolic leaf extract of *Wedelia chinensis* were presented in Table 2. From the table, it can be predicted that the amount of alkaloids, flavonoids, phenols and tannins of methanolic leaf extract of *Wedelia chinensis* were found to be 5.2 mg/gm, 5.7 mg/gm, 6.6 mg/gm and 4.8 mg/gm respectively.



Fig. 1: Aerial parts of the plant *Wedelia chinensis* (Osbeck) Merrill

Table 1: Preliminary phytochemical tests for the presence of active constituents in *Wedelia chinensis*

S. No	Compounds	Petroleum ether	Chloroform	Ethyl acetate	Methanol	
1.	Alkaloids	Dragendorff reagent	-	+	+++	
		Meyer's reagent	-	+	+++	
		Wagner's reagent	-	+	+++	
2.	Flavonoids	-	+	+	+++	
3.	Saponins	-	-	-	-	
4.	Phenols	Ferric chloride test	-	+	+	+++
		Lead acetate test	-	+	+	+++
		Liebarmann's test	-	+	+	+++
5.	Steroids	Liebermann-Burchard's test	-	-	-	
		Salkowski reaction	-	-	-	-
6.	Glycosides	-	-	-	-	
7.	Tannins	Lead acetate test	-	+	+	+++
		Ferric chloride test	-	+	+	+++
8.	Cardiac glycosides	-	-	-	-	
9.	Anthraquinones	-	-	-	-	
10.	Resins	-	-	-	-	

- not present, + - low presence, +++ - high presence

Table 2: Quantitative phytochemical analysis showed the presence of bioactive compounds in the methanolic leaf extracts of *Wedelia chinensis*

S.No	Bioactive compounds	Quantity
1.	Alkaloids	5.2 mg/gm
2.	Flavonoids	5.7 mg/gm of Rutin
3.	Phenols	6.6 mg/gm of Gallic acid
4.	Tannins	4.8 mg/gm of Tannic acid

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