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# A comprehensive investigation of phytochemical and *in vitro* biological potential of petroleum ether fraction of *Wedelia trilobata* leaves

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**Abstract**
*Wedelia trilobata* leaves in Asteraceae's plant family have an ethnomedicinal effect and treat inflammation and infection. We aim to investigate the phytochemical and biological properties of this plant's Petroleum Ether fraction of leaf extract. The total flavonoid content determination assay was used to investigate the antioxidant properties. The disk diffusion method ascertained the antibacterial activity, and the cytotoxic activity was assessed using a brine shrimp mortality experiment. The phytochemical evaluation of the petroleum ether fraction of *W. trilobata* leaves showed higher flavonoid contents (727.64 mg of AAE/gm in 1gm/ml of extract). The extract showed remarkable cytotoxicity with maximum effect at 400 µm/ml concentration ($LC_{50}$=15.28 µg/ml). The extract showed lower antimicrobial activity when compared to Ciprofloxacin. The Petroleum Ether fraction of *W. trilobate* leaves exhibited potent cytotoxic activity, and it can further evaluate anticancer, pesticidal, antitumor, and other pharmacological activity.

**Keywords:** *Wedelia trilobata,* phytochemicals, antioxidant, cytotoxic, antimicrobial

## 1. Introduction
Medicinal plants serve humankind as an essential source of drugs and foods worldwide. Today, a sizable portion of the global population uses plant-based therapeutics as either their own "traditional medicine" or "complementary and alternative medicine" to address their health needs. [1-4]. For example, by selling herbal supplements, an estimated 8 billion USD was earned in the US in 2017, with an increase of 8.5% compared to previous years [5]. Natural plants as medicinal agents started in ancient times and led to modern pharmaceuticals' discovery to control various diseases [6]. The scientific exploration of pharmacologically active constituents in medicinal plants shows that the general public's interest in natural medicinal plants has increased significantly [7]. Over 1000 bioactive compounds have been extracted from numerous herbal plants [8]. Alkaloids, flavonoids, tannins, and phenolic substances are the most common and emergent among these bioactive constituents, providing many clinical advantages [9].
About 70% of fatalities are mostly brought on by infectious diseases caused by bacteria, fungus, or viruses.[10-12]. To fight against the rapidly increasing drug resistance of bacteria and fungi, ethnopharmacologists, botanists, chemists, and other natural product analysts are trying to extract pharmacologically active phytochemicals from the available medicinal plants. Since antibiotic resistance has become one of the most serious risks to the health of the general people and available antibiotics are positively associated with hypersensitivity and allergic reactions, researchers are trying to develop novel natural antimicrobial drugs to fight against these adverse reactions [10-12].
Numerous diseases, including ageing, cancer, diabetes, atherosclerosis, arthritis, Alzheimer's disease, and many other age-related conditions, are caused by free radicals. [13-15]. Sometimes, these may trigger apoptosis (programmed cell death) and necrosis [16]. According to scientific studies, antioxidants can decrease free radicals' activity and side effects and enhance cell survival times effectively [13,17]. In 2015, there were 8.7 million cancer-related deaths worldwide and about 17.5 million new instances of the disease [18]. The development of safer and more potent chemical therapies was necessitated by the anti-cancer medications' severe side effects and low selectivity [19]. The lethality bioassay for brine shrimp is a quick, effective, and affordable test for identifying bioactive compounds and determining their cytotoxic activities [20].

One type of Asteraceae species, *Wedelia trilobata* Linn, AS Hitchc., is referred to as Bhringraj in Bangladesh [21] and has been used as traditional herbal medicine. Several studies isolated main bioactive compounds such as sesquiterpene lactones, trilobolid-6-O-isobutyrates A and B (from the flower); diterpene (kaurenoic acid), eudesmanolide lactones, and luteolin (from leaves and stems); and other constituents like tannin, saponins, flavonoids, phenol, terpenoids [22 ,23]. It has historically been used for cold treatment and flu, fever, sores, inflammation, hepatitis, infections, amenorrhea, dysmenorrhea, reproductive problems, and clearing the placenta after birth [24]. It has been discovered that *W. trilobata* may have potential benefits for wound healing, uterine contraction, diabetes, hepatoprotection, antioxidant, antibacterial, anticancer, anti-inflammatory, and analgesic properties. [25]. Based on this potential evidence, we carried out this exploratory study to analyze preliminary phytochemical and biological properties such as antimicrobial, cytotoxicity, and antioxidant potential of petroleum ether fraction of *W. trilobata.*

## 2. Methods

### 2.1 Plant material collection and processing

Fresh leaves of the *Wedelia trilobata* plant were collected from Aftabnagar, Dhaka, Bangladesh. An expert taxonomist from Bangladesh National Heberium, Mirpur, Dhaka, Bangladesh, carried out proper identification of the plant sample (Accession number- 38512). The plant leaves were sun-dried for a few days. The plant materials were dried for a whole day at a significantly lower temperature to improve grinding. Next, the dried leaves were pulverized in the Phytochemical Research Laboratory into a coarse powder using large-capacity grinding machines at the Department of Pharmacy, East-West University, and Bangladesh.

### 2.2 Preparation of crude extracts and fractions

A separate, spotless, five-litre flask with a circular bottom was filled with about 650 grams of the powdered leaves, which were then soaked in 3.5 litres of methanol. For fifteen days, the container was kept with sporadic shaking and stirring. After filtering the entire mixture, the resulting filtrate was concentrated using a rotary evaporator at 39°C. After air-drying the concentrated extract to a solid residue, 5 grams of methanol extract were added with 100 mL of 90% methanol to prepare stock extract solution. The mother solution was redissolved in 250 mL of distilled water to obtain petroleum ether dissolved fractions and divided into fractions of different polarity using an equal volume of petroleum ether. The petroleum ether soluble fraction was collected separately and allowed to air dry following the partitioning of the mother solution.

### Antioxidant activity

#### 2.2.1 Total flavonoid content

The amount of flavonoid in the petroleum ether fraction was determined by comparing the reaction mixture's absorbance at 510 nm with a UV-visible spectrophotometer against a blank solution. The reference standard employed in this study was ascorbic acid (AA) at varying concentrations [26]. 6 mL of purified water was added to a test tube containing 1.5 mL of extracts. After that, 5% NaNO2 was added and left to incubate for six minutes. The next step was to add 10% $AlCl_3$ and incubate for six minutes. Lastly, 0.6 mL of distilled water and 4% NaOH were added and incubated at room temperature for 15 minutes. 1.5 ml of petroleum ether was used for the blank solution, and an identical process was carried out. Next, using a spectrophotometer to measure the solution absorbance against the blank was calculated at 510nm.

Flavonoid extract + $AlCl_3$ = Flavonoid-aluminium complex ($\lambda_{max}$510nm)

### 2.3 Brine shrimp lethality bioassay (BSLA)

The broad spectrum of pharmacological activity and cytotoxicity of natural compounds are indicated by the BSLA [27, 28]. The procedure for performing this bioassay was as outlined by Meyer *et al.* [29]. After dissolving 38 grams of pure NaCl in 3.8 grams of distilled water, the prepared brine eater was mixed with the dry preserved eggs of *Artemia salina* Leach. The eggs were incubated at room temperature (25-30ºC) for 18-24 hours under constant illumination and aeration for survival and maturity.

Dissolve 4 mg of the material in 200 µL of pure DMSO to make the stock solution. The 400 µg/mL to 0.78125 µg/mL concentrations were then obtained using serial dilution for ten dilutions. Then, using a pipette, ten live shrimp were gathered and put to the test solutions. Three attempts at the tests were made. The negative control was made with 100 µL of DMSO added to a test tube containing 10 nauplii and 5 ml of seawater, whereas the positive control was made with tamoxifen (20 µg/mL). At 25 °C for 24 hours, the number of dead larvae was counted, and the percentage of mortality was calculated.

### Antimicrobial activity

The diameter of the zone is correlated with the susceptibility of the isolate and the pace at which the medication diffuses through the agar media [30]. The pure cultures of the bacterial strains utilized in the experiment were obtained from the microbiology lab at East-West University. The test organisms included fungus (*Saccharomyces cerevisiae)*, bacteria (Gram-positive: *Beta-hemolytic streptococcus, Bacillus subtilis*; Gram-negative: *Escherichia coli, Salmonella paratyphi, Pseudomonas aeruginosa*).

5.6gm of agar medium was added to distilled water to make 200 mL to prepare the medium and then autoclaved to make a clear solution. The test species were subsequently moved into a Petri dish containing approximately 10 ml of sterilised and melted agar. Stersterilized metrical filter disks (BBL, Cocksville, U.S.A.) have been taken under a laminar hood in a petri dish. After that, test sample solutions were soaked into the discs and dried. Standard Ciprofloxacin discs were employed as positive controls, whereas petroleum ether discs were negative controls. Sample discs, standard antibiotic discs, and control discs were carefully placed on the previously defined zones on the agar plates that had been pre-inoculated with test bacteria for diffusion and incubation. After that, they were turned upside down and kept in a 37°C incubator for 24 hours. Finally, the diameter of the inhibition zones using a clear scale allowed us to determine the antibacterial activity of the test compounds.

### 2.4 Statistic calculation

All the *in vitro* experiment results were expressed as linear diagrams and regression analysis was calculated in Microsoft Office Excel 2013.

## 3. Results

Using AA as the reference standard, the petroleum ether fractions of *W. trilobata* leaves were treated to ascertain the

total flavonoid concentration present. Plotting the absorbance versus concentrations revealed a linear association after AA concentrations ranging from 50µg/ml to 250µg/ml were measured. The total flavonoid content of the extract was determined in mg/ml using the standard curve regression equation. The total flavonoid content in pet. Ether fraction of *W. trilobata* (leaves) was 727.647 mg of AAE/gm of dried extract. It follows that this extract has antioxidant properties.

In the Brine Shrimp mortality Bioassay, exposure to various quantities of test substances resulted in diverse degrees of mortality. The lethality degree was proportionate to the concentration in both the standard and Petroleum Ether fraction samples. The Comparative assessment of mortality (%) of Tamoxifen (Standard) and Pet ether extract a fraction of *W. trilobata* leaves treated group against different concentrations is represented in Figure-1. The death rate progressively rose as the test sample concentration rose. The highest concentration at 400 µg/ml resulted in the greatest number of deaths. The calculated $LC_{50}$ values petroleum ether fraction and the standard in this investigation were 15.28 µg/ml and 13.38 µg/ml, respectively. The $R^2$ values are closer to one for both the Pet. ether fraction and the standard, indicating that the extract has strong activity against brine shrimp nauplii that is equivalent to the standard (Tamoxifen). Figure 2 shows that the lethal levels required for killing 50 percent of the sample population are close to the standard for an aqueous fraction. So, Petroleum ether fractions of *Wedelia trilobata* leaves have potential cytotoxic activity.

Pet. ether fraction of methanolic extract of *W. trilobata* showed low antimicrobial activity compared to Ciprofloxacin, as shown in Table 1. None of the inhibition zones of the pet. Ether fraction is equal to Ciprofloxacin against any bacteria or fungi. The best antimicrobial activity was found against *Aspergillus Niger* (8mm) compared to the standard (30mm) among all the microbiological cultures.

## 4. Discussion

Exploring medications from natural resources is paramount in drug discovery and development. Nature has been a prolific source of bioactive compounds that have formed the basis of numerous drugs. The complexity and diversity of natural substances offer a vast pool of potential therapeutic agents, many of which possess unique structures and mechanisms of action. A previous study on *W. trilobata* showed it has anti-inflammatory activity [31]. Pharmacological reports on this plant revealed potential anti-inflammatory, analgesic, wound healing, antioxidant, Hepatoprotective, larvicidal, trypanocidal, antidiabetic, and within the treatment of female reproductive system disease [23]. W. trilobata has great promise for detailed pharmacological activity tracing, including effects on microorganisms, inflammation, and reproductive functions. Through this exploratory work, we attempted to highlight W. *trilobate's* untapped potential, which could help create new formulations with greater therapeutic benefits.

The phytochemical screening of Pet. Ether fraction of *Wedelia trilobata* leaves exhibited a significant presence of flavonoid content, i.e., 727.647 mg of AAE./gm in 1gm/ml of extract. One possible explanation for the antibacterial effects of active acetone extract could be the presence of potential phytochemicals such as phenolics, flavonoids, and terpenoids [32]. The main components of the essential oil, limonene and α-pinene, were found to have modest functions in antioxidant biological tests. The essential oil (1000 µg/disc) showed promising antibacterial activity against ten strains of microorganisms as measured by the MIC values (125 to 250 µg/ml) and the diameter of the zones of inhibition (20.8 to 22.2 mm). The antibacterial activity of α-pinene was found to be greater than that of the volatile oil, as evidenced by its MIC values (62.5 to 125 µg/ml) and inhibition zone diameter (20.7-22.3 mm). Volatile oil's major and minor synergistic components may also be responsible for its antioxidant and antibacterial activities.[33].

In the case of both standard and petroleum ether fraction samples, it was discovered that the degree of lethality was proportionate to the concentration. The greatest concentration of 400 µg/ml resulted in the highest mortality rates for the species, while the lowest concentration produced the lowest mortality rates of 0.78125 µm/ml. Grandiflorenic acid demonstrated the ability of the ethyl acetate fraction of the ethanolic extract of *W. trilobate* leaves to heal wounds. On the other hand, Grandiflorenicacid (2.5µg/mL) resulted in the percentage viability of Bhuman fibroblast, keratinocytes 116%, and 106%, respectively, the scientific evidence of potential wound healing activity of grandiflorenic acid. This activity is made possible by fibroblast stimulation in conjunction with inhibition of prolonging the inflammatory phase of wound healing, as demonstrated by lower levels of inflammatory cytokines released from macrophage Raw 264.7 cells [22, 23].

When compared to Ciprofloxacin, the extract of *W. trilobata* showed lower antimicrobial activity than Ciprofloxacin. The highest antimicrobial activity was found against *Aspergillus Niger* (8mm), comparable to the standard (30mm) among all available microbial cultures. Ethyl acetate extract was active only in *Salmonella* Group C, and against tested bacteria, the extract was inactive [34]. Although pet. Ether fraction of *W. trilobata* showed lower antibacterial activity, and n-hexane extracts showed activity against *some* gram-negative bacteria [34]. No extracts tested showed biological activities against yeasts or fungi [34]. Although we cannot determine the cytotoxic activity on the cancer cell line, there might be a possibility of anticancer potential of pet. Ether fraction of *W. trilobata* leaves extract. It is imperative to conduct additional research on discovering and purifying bioactive chemicals.

**Table 1:** Antimicrobial activity of the standard sample (Ciprofloxacin) and Pet. ether fractions of the methanolic extract of *Wedelia trilobata* leaves

| Type of microorganism | | Zone of inhibition (mm) | |
|---|---|---|---|
| | | Standard sample | Pet. ether fraction |
| Gram-Positive Bacteria | *Bacillus cereus* | 38 | 8 |
| | *Bacillusmegaterium* | 38 | 7 |
| | *Bacillus subtilis* | 40 | 6 |
| | *Staphylococcus aureus* | 40 | 8 |
| | *Sarcina lutea* | 37 | 6 |
| Gram Negative Bacteria | *Salmonella paratyphi* | 38 | 6 |
| | *Salmonella Typhi* | 36 | 7 |

| | | | |
|---|---|---|---|
| | *Vibrio parahemolyticus* | 40 | 7 |
| | *Vibrio parahemolyticus* | 40 | 7 |
| | *Escherichia coli* | 36 | 5 |
| | *Vibrio mimicus* | 35 | 8 |
| | *Shigella dysenteriae* | 38 | 8 |
| | *Pseudomonas aeruginosa* | 38 | 6 |
| | *Shigella boydil* | 48 | 6 |
| Fungi | *Saccharomyces cerevisiae* | 35 | 6 |
| | *Candida albicans* | 26 | 6 |
| | *Aspergillus niger* | 30 | 8 |

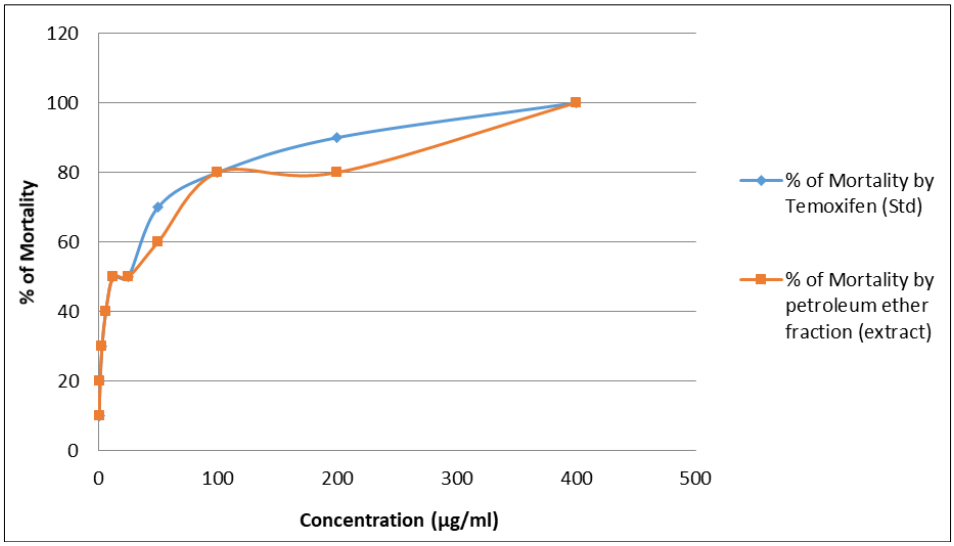


**Fig 1:** Comparative assessment of mortality (%) of Temoxifen and Pet ether extract fraction of *Wedelia trilobata* leaves treated group against different concentration

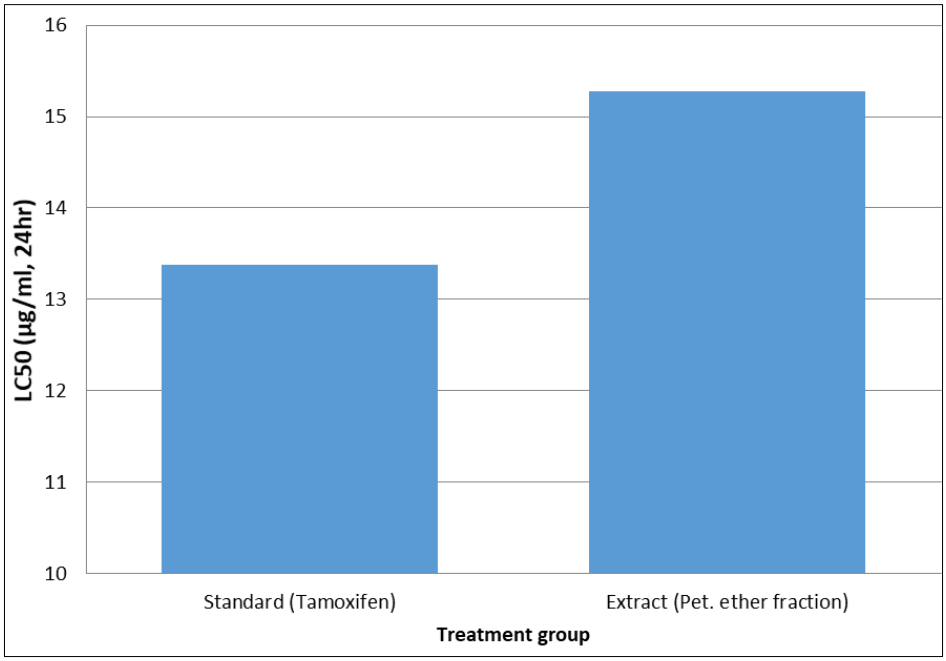


**Fig 2:** Comparison between $LC_{50}$ values of standard and Petroleum ether fractions of *Wedelia trilobata* leaves extract

## 5. Conclusions

The findings demonstrate the strong cytotoxic activity of the petroleum ether fraction of *W. trilobata* leaves. According to experimental analysis, this plant's leaves also have antibacterial properties and contain flavonoids, which act as antioxidants. The leaves of *W. trilobata* showed strong cytotoxic activity; therefore, more research on the leaves' antitumor, pesticidal, and anticancer effects is warranted. To produce innovative medications, thorough research can be done to isolate and identify the active chemicals in the leaf extract that are responsible for this pharmacological activity.

## 6. List of Abbreviations

BSLA: The brine shrimp lethality assay
DMSO: Dimethyl Sulfoxide
SEM.: Standard Error Mean
AA: Ascorbic Acid
MIC: Maximum Inhibitory concentration

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