Research Article

Antibacterial and Antifungal Activities of the Medicinal Plant Veronica biloba

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Plants are naturally God gifted for the synthesis of medicinal compound and provide a great help in a new discovery in the area of chemical diversity because of the unknown availability either as a standardized extract or as a pure compound. The medicinal plant Veronica biloba extracts obtained through Soxhlet and maceration methods were subjected to preliminary antimicrobial screening against pathogenic microorganisms. Fractionation was performed using liquid-liquid extracts such as ethyl acetate, water, dichloromethane, and hexane extract of plant, and the fractions were tested for antifungal activity and antibacterial activity using well-diffusion method at sample concentration of 10–30 μL. The result indicated that all extracts exhibited antimicrobial activity against all test pathogens. The ethyl acetate extract showed greater activity than other corresponding extracts. Among various extracts, only the ethyl acetate extract show potential against bacterial (gram negative and gram positive) and fungus test strain greater than standard Nystatin test control. Thus, the extract of Veronica biloba could be used to treat microbial (fungus and bacterial strain) infection.

1. Introduction

Plants are naturally God gifted for the synthesis of medicinal compound. Their isolation from medicinal plants and the characterization of the active compound they contain provide a great help in the preparation of new drugs to treat many diseases and have a high therapeutic value [1]. The plant extract, which is also called natural product, provided a great help in a new discovery in the area of chemical diversity because of the unknown availability either as standardized extract or as pure compound [2]. According to pharmaceutical studies, approximately 10 to 20% of plants are used in a positive way in health care to treat harmful diseases such as cancer [3]. The classical example is reported on the bark of yew tree, which mainly contains taxol and is used in ovarian cancer and breast cancer [2]. Isolation or extraction of medicinal plants mainly produced one or several substances that are responsible for any activity and are closely related to each other [4]. Plants are the main source of drugs in modern medicinal system, folk medicinal system, traditional medicinal system, food supplement, and for synthetic drug [5]. A recent research study shows that medicinal plants show mainly antioxidant activity. The phenolic compounds such as flavonoids, lignins, and vitamins A, C, and E, and tannins all are antioxidants and are present mainly in plants [6]. The people interested in conventional medicine called drugs from plant because of some reason they are efficient, effective therapy, and have no side effects, while ecological awareness show that natural products are harmless. Whereas wrong use or abusive use of synthetic drugs cause many problems and have many side effects [7]. The Veronica (Plantaginaceae) genus consisting of about 450 known species exists in both hemisphere and temperate region [8]. They have 79 popular species of which 26 are endemic [9]. Due to the great importance of Veronica species throughout the world, they are selected for our investigation.
2. History

According to the report of the World Health Organization, about 80% people used traditional medicine for primary health care treatment. In Asia, plants as medicine show long history with human involvement in the environment. Herbal medicines contain different types of novel and unique substances to treat infectious and chronic diseases [12]. The tradition of using plant products to treat a number of diseases starts with the beginning of human civilization. The earliest document shedding light on the use of medicinal plants is Hindu Culture, written between 4500 and 1600 BC [13]. The use of traditional medicine or natural products is oldest as with the human civilization medicine from plants has therapeutic properties and history write that from long time the main source of drug was plants, minerals, and animal products [14]. The synthetic chemical drugs show bad health-related side effects, microbial resistance man tend to ethnopharmacognosy obtained thousands of phytochemical from plant with less or, no side effect, safe and mainly effective with many biological activities such as analgesic, antimicrobial, wound-healing, antioxidant, anticancer, antiarrhythm activities. Some people claim that natural products are beneficial for health. So, clinical trials tend to verify that claim of bioactive part, their formulation, safeguard, and side effects before the drug is provided to the patients. According to the report of the World Health Organization, 12 mega biodiversity countries nearly have 20,000 medicinal plants [15]. The isolation of penicillin from microorganisms is clearly important with the development of anti-infective therapy. Approximately 25% of drugs used throughout the world are obtained from plants. The World Health Organization reported 252 essential active compounds and about 121 are in current use. More than 11% of synthetic drugs are obtained from natural plant source. Some valuable drugs isolated from plants are quinine and quinine isolated from Cinchona, atropine from Atropa belladonna, and codeine and morphine from Papaver somniferum. The clinical trial obtained drug from natural source at least 60% of anti-infective, antitumor, drug available in market [16]. Naturally isolated active part of the plant is important and is used to cure physiological, pharmacological and biochemical study such as phorbol ester, cannabinoids, forskolin, mucaraine, colchicines, and yohimbine [17]. Many of these cannot be yet economically synthesized and are mainly isolated from cultivated or wild plants [18].

3. Field of Knowledge

The research on a plant origin for a therapeutic medicinal material discovery or development is expensive and is a hard task [19]. Developing a new drug requires about 100–360 million US$ and at least ten years’ work on it. Up to 1992, 10,000 compounds were tested, with only 1 of 4 being approved active for drugs. The National Cancer Institute found three biologically active compounds to treat human immunodeficiency virus in 50,000 tested plant extracts and three active compounds for antitumor activity in 33,000 tested plant extracts [17]. They involved the basic knowledge of science, pharmacology, botany, toxicology, and chemistry. These particular disciplines should not be considered as secondary for one another. To account for a medicine from plants, other fields of knowledge, which include organic chemistry, anthropology, biotechnology, agronomy, and a fundamental pharmaceutical, have important roles in the designing any new drug from plants [20]. When a medicinal plant is found, the methods applying for therapeutic treatment (as home-made) are herbal teas or preparation of pharmaceutical powder pills, tinctures, capsules, fluid extract, standard enrich, or crude extract. Finally, a plant that contains active natural compounds that is itself responsible for drug can be isolated and purified by extraction process, such as ergotamine (as a precursor, for example, diogenin), digoxin, and quinine [21].

4. Selection of Plant

The approach for a suitable plant selection is hard and very important. In pharmacology, discussions depend on the requirement to isolate a natural active compound or make a herbal medicine, which involves several roots of traditional usage, toxicity, chemical content, and randomized several requirements [22, 23]. The common cultural medicine is called ethnopharmacology or ethnobotany. It shows how usage of natural folk medicine is highly important and how ethnic groups utilized it, and their procedure of preparation provides information on pharmacological activity and extraction process. A different culture has its own health care system and health illness [24]. Selection of active compounds against insects and bacteria depend on environment of the plant [18]. However, a specific potent therapeutic drug in biological research has been found in a toxic plant [17]. For a pharmacological activity, certain plant families and genera are selected based on chemotaxonomic or phylogenetic information [25, 26]. For selection of plants, researchers decide a randomized search for active pharmacological species; for example, discovery of an antitumor drug follows this strategy and for choosing a selective plant, study scientific literature or, if identified, find a new way [23, 27, 28]. A cultivated plant usually selected can provide genetically guarantying homogeneous material with extinction threatened species [29]. In recent few years in the research area, a number of publications studied biologically active plant-derived compounds that are anti-inflammatory, antibiotic, antitumor, contraceptive, and kidney medication and for psychiatric treatment. However, a priority is shown towards a viral, cardiovascular, and tumor diseases [18]. A taxol naturally active compound diterpenes show anti-tumor activity obtained from Taxus. About 2500 mg taxol isolation required more than 12,000 trees to be cut down and 27,000
tons of *T. bacata* and *T. brevifolia* bark was obtained. Due to high requirement of *Taxus*, it is necessary to find alternative sources in other plants or should be synthesized in a considerable amount [30].

5. Experimental Methodology

5.1. Identification of Plant. The medicinal plant species *Veronica biloba*, biolobed, two-lobed speedwell, of genus *Veronica* was identified and confirmed with the help of botanical expert Prof. Muhammad Israr of Botany Department, Govt. Post Graduate College Mardan, and also through various literature survey comparisons.

5.2. Collection of the Plant. The medicinal plant used in the project/experiment was the whole plant selected. Fresh whole plants in their flowering stage were collected from Sang-e-mar mar, Near Par Hoti District Mardan, and also from Surkh Dheri, Rustam, Mardan. The plant collection was done during the month of February–March. Healthy plants are collected/selected from a fertile land.

5.3. Drying and Grinding of the Plant. After cleaning of the collected plants, they were cut into small pieces by using knives and scissor. They were stored for drying under shade, and to avoid/protect from surrounding contamination and dust present in the environment. The drying was done in a room for about two weeks (2-week), without any exposure to light. After completely drying the plants, obtain uniform-sized powder and ensure to enhance the surface area for better extraction process.

5.4. Extraction

5.4.1. Soxhlet Extraction. 30 g of finely ground uniform-size powder of the plant sample is kept in a thimble, a porous bag made from cellulose strong filter (paper prepared manually), and then thimble is inserted into thimble chamber of Soxhlet. Extraction was carried out in 300 ml ethanol kept in the bottom flask of Soxhlet. The upper part was fitted with a condenser by introducing water inflow and outflow. The solvent was heated at moderate temperature around 40°C over mantox heater, and the solvent vaporizes and goes to sample thimble chamber, condenses, and falls back when the liquid extract reaches the siphon arm and emptied into down a bottom flask again and again. The process was continued for 48 hrs until solvent drop cannot leave residue when evaporated. Furthermore, fractionation is carried out on water, dichloromethane, *n*-hexane, and ethyl acetate. The four fractions were then concentrated to get dried extract for further analysis of biological activities.

5.4.2. Maceration. In this method, 20 g grinded powdered plant sample is kept in a closed jar (made from Pyrex glass), and 200 ml absolute ethanol is added. The jar is allowed for up to 3 weeks at room temperature, and proper shaking is performed on a daily basis to release plant-soluble phytochemicals. The extract obtained via soaking is filtered through a normal filter paper (Whatman filter paper) to get concentrated ethanolic extract with evaporation of the solvent. Both the extracts were analyzed by thin-layer chromatography (TLC) to confirm their similarity pattern if any. Furthermore, same fractionation was done as for above Soxhlet fraction obtained in water, dichloromethane, *n*-hexane, and ethyl acetate. A fraction was then concentrated to obtain the desired dry extract for further analysis of biological activities.

5.5. Antibacterial Activity

5.5.1. Preparation of Fraction Extracts Solution. The dried four fractions obtained were dissolved to make a solution of concentration in dimethylsulfoxide (DMSO) of 20 mg/ml. For proper mixing, the solution was kept in centrifuge for 25 minutes at 13000 rpm. The standard antibiotics gentamicin (10 mg/discs), ampicillin (10 mg/discs), and ofloxacin (1 mg/ml) were used for comparing the activity with each active fraction.

5.5.2. Microbes Used in the Test. The microorganisms used in the study were obtained from the Microbiology Department of Abdul Wali Khan University Mardan. Gram-negative bacteria used was *Escherichia coli*, and the gram-positive bacteria was *Staphylococcus aureus*.

5.5.3. Culture: Media Preparation. Microorganism suspension was prepared as McFarland standard. For the antibacterial sensitivity test analysis, the MHA (Müller–Hinton Agar) was used for bacterial media preparation. The culture media were prepared in 250 ml distilled water by dissolving 9.5 g of MHA. The obtained amber color solution is mixed thoroughly and boiled with frequent agitation to dissolve agar powder completely and a clear to slightly opalescent gel is obtained. Then, autoclave the media for sterilization at 15 lbs pressure, at 121°C temperature, for 15 minutes. Allow sterilized media to cool at room temperature in laminar flow hood, and then pour 25 ml of the media into each Petri plate and leave for few minutes to allow the media to solidify. After solidification, spread the culture microbes on media by using cotton swab and cover the whole media with turn 90° degree rotation without leaving any gap. Make 6 bores in each Petri plate separated from each other by 2.5 cm distance. 30 μl of each fraction is poured in the first 4 bores, antibiotics in the second last bore, and solvent in the last bore. For positive control, two plates are placed for both microbes with no antibiotics and extract fraction added, whereas for sterility of media negative control, one Petri plate is placed without any microbes. Store all Petri plates for incubation in biochemical oxygen demand (BOD) incubator at 37°C for 24 hrs. Table 1 shows the result of antibacterial activity. The inhibited zone for each fraction and active drug measured are calculated as mean ± standard deviation (SD).
5.6. Antifungal Activity. The antifungal activity was performed on nutrient agar with the fungus *Aspergillus fumigatus* obtained from the Biochemistry department of Malakand University, Chakdara. The culture was done as McFarland standard sterilized media prepared at 121°C for 14 minutes in autoclave. The well-diffusion method was applied as per requirement after streaking cultured for 12–14 hrs. 10 μl of extract fraction was used for activity analysis, 10 μl of Nystatin was used as the standard sample, and 10 μl of oxytetracycline was used as the test control. Store all Petri plates for an incubation period of 72 hrs at temperature 20°C. Table 2 shows the result of antifungal activity. Inhibited zone was calculated for each fraction as mean ± SD (standard deviation).

6. Results and Discussion

The current attempt was made due to resistance development in bacteria and fungi (microbes) to available drugs. Agar well-diffusion method was used for antimicrobial screening using a standard protocol of clinical laboratory prescribed by national committee [31]. Extracted plant antibiotics are safe, effective, and have no or little side effects [32]. The active phytochemicals are responsible for biological activity such as antimicrobial against pathogens provide help in discovery of new antibiotic drugs [33–35]. The present study investigated the antimicrobial and antifungal potential of a medicinal plant *Veronica biloba* for the first time.

The antibacterial report of the medicinal plant *Veronica biloba* fractionation extracts is summarized in Table 1. The *Veronica biloba* extracts show dose-dependent potential activity and affect the tested pathogens. The crude ethyl acetate extract is more potent (shown in Figure 1 and Table 1) against both bacterial strains *Staphylococcus aureus* and *Escherichia coli*. The *Veronica biloba* ethyl acetate extracted fraction showed 10.5 ± 1 mm maximum zone of inhibition at 30 μl concentration with *S. aureus* and 7.3 ± 0.2 mm at 30 μl with *E. coli* (shown in Table 1). However, the aqueous extracted fraction showed 5.1 ± 0.2 mm and 4.5 ± 0.5 mm inhibited zone with *S. aureus* and *E. coli*, respectively, at 30 μl. The hexane extracted fraction showed 6.3 ± 0.5 mm and 4.3 ± 0.2 mm zone of inhibition with *S. aureus* and *E. coli*, respectively, which is comparatively less than that using the ethyl acetate fraction (shown in Figure 1). Dichloromethane fraction showed less activity with *S. aureus* 4.3 ± 0.2 mm and 6.3 ± 0.5 mm with

### Table 1: Result of antibacterial activity of *Veronica biloba*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (μl)</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>30 μl</td>
<td>4.3 ± 0.2 mm</td>
<td>6.3 ± 0.5 mm</td>
</tr>
<tr>
<td>Water</td>
<td>30 μl</td>
<td>5.1 ± 0.2 mm</td>
<td>4.5 ± 0.5 mm</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>30 μl</td>
<td>4.5 ± 0.5 mm</td>
<td>6.8 ± 0.2 mm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>30 μl</td>
<td>10.5 ± 1 mm</td>
<td>7.3 ± 0.2 mm</td>
</tr>
<tr>
<td>Ampicillin (+control)</td>
<td>10 mg</td>
<td>15.5 ± 0.3 mm</td>
<td>—</td>
</tr>
<tr>
<td>Gentamicin (+control)</td>
<td>10 mg</td>
<td>—</td>
<td>11.9 ± 0.4 mm</td>
</tr>
<tr>
<td>Ofloxacin (+control)</td>
<td>1 mg</td>
<td>20 ± 0.5 mm</td>
<td>11.5 ± 0.15 mm</td>
</tr>
<tr>
<td>DMSO (−control)</td>
<td>30 μl</td>
<td>1 ± 0 mm</td>
<td>1 ± 0 mm</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SD (standard deviation) in mm of zone of inhibition shown by each fraction. Compared using ANOVA, with significance level set at alpha of 0.05.

### Table 2: Result of antifungal activity of *Veronica biloba*.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Concentration (μl)</th>
<th><em>Aspergillus fumigatus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethane</td>
<td>10 μl</td>
<td>8.3 ± 0.5 mm</td>
</tr>
<tr>
<td>n-Hexane</td>
<td>10 μl</td>
<td>12.1 ± 0.2 mm</td>
</tr>
<tr>
<td>Water</td>
<td>10 μl</td>
<td>10.6 ± 0.5 mm</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>10 μl</td>
<td>12.3 ± 0.5 mm</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>10 μl</td>
<td>26 ± 0 mm</td>
</tr>
<tr>
<td>Nystatin (standard)</td>
<td>10 μl</td>
<td>6.7 ± 0.5 mm</td>
</tr>
<tr>
<td>DMSO (negative control)</td>
<td>10 μl</td>
<td>1 ± 0 mm</td>
</tr>
</tbody>
</table>

The data are expressed as mean ± SD (standard deviation) in mm of zone of inhibition shown by each fraction. Compared using ANOVA, with significance level set at alpha of 0.05.

![Figure 1: Inhibited zone shown by each fraction of *Veronica biloba* against bacterial strain.](image)

*E. coli* zone of inhibition as compared with the standard antibiotics ofloxacin 11.5 ± 0.15 mm, ampicillin 11.9 ± 0.4 mm less inhibition causes, while gentamicin 15.5 ± 0.3 mm, showed more zone of inhibition (shown in Figure 1 and Table 1).

The antifungal assay result of *Veronica biloba* is summarized in Table 2. The crude extracted fractions of *Veronica biloba* strongly inhibited the fungus *Aspergillus fumigatus* in the same concentration. The maximum inhibition was shown by ethyl acetate extract 12.3 ± 0.5 mm zone of inhibition at 10 μl concentration (shown in Figure 2). However, hexane extract cause 12.1 ± 0.2 mm, water
Antifungal activity

Fractons activity against fungus strain

DCM
Hexane
Et-acetate
Water
Nystatin
Oxytetracycline

Figure 2: Inhibited zone shown by each fraction of Veronica biloba against fungus strain.

10.6 ± 0.5 mm, and dichloromethane 8.3 ± 0.5 mm inhibition at 10 μL concentration. The standard nystatin (test control) show at 10 μL concentration 6.7 ± 0.5 mm less zone of inhibition as compared to a medicinal plant Veronica biloba extracted fractions (shown in Figure 2 and Table 2).

Table 2: Fractions activity against fungus strain

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DCM</td>
<td>7.2 ± 0.5</td>
</tr>
<tr>
<td>Hexane</td>
<td>8.3 ± 0.5</td>
</tr>
<tr>
<td>Et-acetate</td>
<td>10.6 ± 0.5</td>
</tr>
<tr>
<td>Water</td>
<td>15.0 ± 0.5</td>
</tr>
<tr>
<td>Nystatin</td>
<td>20.0 ± 0.5</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>25.0 ± 0.5</td>
</tr>
</tbody>
</table>

Antifungal activity

Veronica montana species. Stojkvoic et al. [55] reported potent antibacterial agent in the extract of Veronica montana species. Exarchou et al. [56] confirmed the antibacterial activity of Veronica extracts through the presence of a hispidulin compound. Dunkic et al. [57] found antimicrobial compounds in Veronica spicata species of this genus.

Phytochemicals, for example, flavonoids, polyphenols, saponins, steroids, tannins, terpenoids, and alkaloids, are natural active compounds present in plants, which are significantly used to treat diseases and also used as nutrient and dietary supplement [58–62]. Flavonoids are mainly polyphenol, and their presence can increase the antibiotics potential against microbes [63, 64]. The flavonoids form complexes with cell wall of bacteria protein and extracellular components and are very important and effective antimicrobial compounds [65]. Terpenoids are involved in weakening microorganism cell wall and membranous tissue dissolution [66]. The interaction of saponins with microbes causes enzyme protein leakage from the cell [67]. Steroid in antimicrobials is responsible for liposome leakage from lipid bilayer membrane [68]. This is the first antimicrobial report on a medicinal plant Veronica biloba against E. coli, S. aureus, and Aspergillus fumigatus pathogens that possesses an extensive useful activity.

7. Conclusion

We have concluded that the extract of Veronica Biloba could be used to treat microbial (fungus and bacterial strain) infection. It can be used either in combination with traditional medication or used alone as an antibiotic.

The fraction extracts of the medicinal plant Veronica biloba (i.e., water, dichloromethane, n-hexane, and ethyl acetate) show both antibacterial and antifungal biological activities in a wide range. The ethyl acetate and n-hexane extracts fraction show more potential in both activities. The change in concentration, purification, and isolation of these extracts can provide us a more sustainable result. Thus, Veronica biloba is a useful medicinal plant and its further assessment is important, which can provide help in the discovery of new antibiotic drug development in the market.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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