Phytochemical Screening, Antioxidant, Total Phenolic Contents, Cytotoxic and Phytotoxic Potential of the Methanolic Extract of *Cornus macrophylla* Wall Leaves


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**Abstract:** Phytochemical screening of the methanolic extract of *Cornus macrophylla* leaves (MECML) revealed various classes of phytochemicals. MECM demonstrated a concentration dependent antioxidant activity, in DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Maximum activity (69.08%) was observed at 100 µg/ml, with IC₅₀ 57.87 µg/ml. Total Phenolic contents (TPC) were determined by Folin-Ciocalteu reagent (FCR) assay and was found 97.3±2.0 mg of gallic acid equivalent per gram of dry weight of the plant material. MECML exhibited profound cytotoxic potential (91%, 1000 µg/ml) against brine shrimp’s nauplii and was directly proportional to the concentration (LC₅₀ 200 µg/ml). Similarly MECML displayed the phytotoxic activity in a dose dependent manner (LD₅₀ 24 µg/ml) and has shown remarkable phytotoxic potential, 95%±0.8 against *Lamina minor* at 1000 µg/ml. Thus on the basis of our findings one can conclude that the methanolic extract of *C. macrophylla* leaves is a rich source of antioxidants and natural herbicides.

**Key words:** *Cornus macrophylla* • Phytochemicals • Antioxidant • TPC • Cytotoxic • Phytotoxic

**INTRODUCTION**

Medicinal plants have been used in different cultures of the world as remedies for human diseases. Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines [1-2]. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [3].

According to the world Health care Organization (WHO) up to 80% of the population in Africa depends on traditional herbal medicine for primary health care [4]. Medicinal plants exhibits various pharmacological, such as cytotoxic, phytotoxic and insecticidal activities [5]. Numerous studies have shown that aromatic medicinal plants are sources of different nutrient and non nutrient molecules, many of which display antioxidant and antimicrobial properties which can protect the human body against both cellular oxidation reaction and pathogen [6]. Thus it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential.

Phytochemicals are naturally occurring compounds synthesized by medicinal plants. Flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action [7]. Besides, phenolic compounds, flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory and anti-carcinogenic etc [8].

*Cornus macrophylla*, Wall is a key member of Cornaceae family. This is a deciduous tree, which normally grow up to 15 meter height. The fruits of *C. macrophylla* are the active ingredient of some of the traditional medicines used for the improvement of liver and kidney function [9]. Furthermore it is also used for the treatment of infections, inflammation and cancer.
In our previous research work we explored the antioxidant and phytotoxic activities in the methanolic extracts of the bark of *C. macrophylla*. Thus looking to the previous result we designed the current study to determine the Phytochemicals, total phenolic contents, antioxidant, cytotoxic and phytotoxic potential of the methanolic extracts of the leaves of the same plant.

**MATERIAL AND METHODS**

**Collection of Plant Material and Identification:** The fresh leaves of *Cornus macrophylla* were collected from the Hazarnau hill of Kot Manzary Baba, District, Malakand agency Khyber Pakhtunkhwa (KPK) Pakistan during June 2014. The plant were identified by Professor Dr. Nasrullah in the Department of Botany, University of Malakand, Khyber Pakhtunkhwa, Pakistan. Voucher specimen was deposited to the herbarium of the same Department.

**Extraction:** After collection, the plants leaves were washed with water, shad dried under room temperature for 10 days. The dried samples were then grounded into a coarse powder with electric blender and stored in polythene bag at room temperature. A portion of the samples was used for detection of different classes of chemical constituents and the remaining plant material was soaked in methanol (80%) for 15 days. After 15 days, the extracts were filtered using Whatman’s No; 1 filter paper. The extract was concentrated to dryness under reduced pressure at temperature of (45°C), using rotary evaporator.

**Phytochemical Screening:** Chemical tests were carried out on crude methanolic extract and powder material of *Cornus macrophylla* leaves using standard procedures to identify the constituents as described by Sofowora, Trease and Evans and Harborne [10-12].

**Alkaloids:** About 0.2 g of the extracts was warmed with 2% H₂SO₄ for two minutes on a boiling water bath. The mixture was then cooled, filtered and treated with the Dragendorff’s reagent. Orange red precipitate indicates the presence of alkaloids.

**Tannins:** The extract (2 g) was mixed with 1 ml distilled water and heated on water bath. The mixture was filtered and 1-3 drops of ferric chloride solution was added to the sample. Then observe the sample for a dark green coloration, which indicates the presence of tannins [10].

**Anthraquinonnes:** Half gram of the extracts was boiled with 2 ml HCl (10%) for few minutes in a water bath. It was filtered and allowed to cool. After cooling, 2 ml of CHCl₃ was added to the filtrate. Then 2-4 drops of NH₃ (10%) were added to the mixture and heat. Formation of rose-pink colour shows the presence of Anthraquinone [10].

**Glycosides:** The extract was hydrolyzed by adding 5 ml HCl solution and neutralized by adding 5 ml NaOH solution. After this a few drops of Fehling’s solution A and B were added to the mixture and observed the sample for red precipitates [11].

**Reducing Sugars:** The extracts (5 g) was shaken with distilled water and filtered. The filtrate was boiled with few drops of Fehling’s solution A and B for few minutes. Then formation of orange red precipitates will confirm the occurrence of reducing sugars.

**Saponins:** About 0.2 g of the sample (Extract) was shaken with 5 ml of distilled water and heated to boil. Then observe the sample for the froth formation (Appearance of creamy miss of small bubbles) [11].

**Phlobutanins:** Half gram was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. After heating the sample were observed for red precipitate [12].

**Flavonoids:** Sample (0.2 g) was dissolved in 2 ml diluted (2%) NaOH and make a solution (a) having yellow colour. After this 2% HCl was added to solution (a) and observe the sample for discoloration.

**Terpenoids (Salkowski test):** The extract (0.2 g) was mixed with 2 ml of chloroform (CHCl₃) and 3 ml concentrated H₂SO₄ was carefully added to form a layer. Then observe a reddish brown colouration of in the interface to indicate positive results.

**Antioxidant Activities**

**DPPH Radical-scavenging Activity:** The hydrogen atom or electron donation abilities of the corresponding methanolic extract and standards were measured from the change of the dark purple-coloured methanol solution of DPPH (1, 1- Diphenyl-2-picryl hydrazyl radical). Experiments were performed according to the method of Yildirim and Blois [13-14] with a slight modification.
Methanolic solution (1 mM) of DPPH free radical was prepared and 1 ml of this solution was mixed with 3 ml of sample solutions (Containing 20-100 µg) and control (Without sample). All the samples were incubated for half an hour and then the absorbance was taken at 517 nm. The lowering of absorbance of DPPH solution indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities (%RSA) was calculated as follow.

\[
\text{%RSA} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{Control absorbance}} \times 100
\]

The assays were carried out in triplicate and the results were expressed as mean values±standard deviations. The extract concentration showing 50% inhibition (EC_{50}) was calculated from the graph of %RSA against extract concentration with quercetin and gallic acid used as standards.

**Determination of Total Phenolic Compounds:** Antioxidant compounds generally contains phenolic group. This assay was carried out as described by Singleton et al [15], with slight modification. Briefly, 1 ml of each of the already prepared extract solution (500 µg) was transferred to 50-ml volumetric flask then volume was adjusted to 45 ml by addition of distilled water. Afterward, 1 ml of Folin-Ciocalteu Reagent (FCR) was added into this mixture and after three minutes 0.3 ml of Na CO$_3$ (2%) was added. Subsequently, the mixture was shaken for 2 h at room temperature and then absorbance was measured at 760 nm. Estimation of the Phenolic compounds was carried out in triplicate. The results obtained were expressed as mg of gallic acid equivalents µg/mg of extract (GAEs) by using the following equation;

\[ A = 1156C + 0.189 \]

where “A” is the absorbance and “C” is the gallic acid equivalent (mg/g).

**Brine Shrimps Lethality Bioassay:** Brain shrimps toxicological assay is a standard protocol for determination of preliminary cytotoxic effect of the test samples. Cytotoxic activity was conducted according to the standard procedure with slight modification [16]. Stock solution of MECML was prepared by dissolving 20 mg of each sample in 2 ml of methanol. Then, 10, 50, 100, 200, 250, 500 and 1000 ppm test doses were prepared in triplicate in separate containers. Solvent (Methanol) was evaporated and 30 brine shrimps nauplii were transferred into each container using a Pasteur pipette. Five milliliters of sea water (Sea salt 38 g/l of distilled water, pH 7.4) was added into each container. One container survived as negative control, 5 ml sea water and 30 shrimps, while etoposied served as positive control. All the containers were incubated at room temperature under ordinary light and the numbers of survived and dead brine shrimps were counted after 24 h. Percent lethality was calculated by applying the following formula;

\[ \text{%Mortality} = \frac{\text{Number of dead brine shrimps nauplii}}{\text{Initial number of live brine shrimps nauplii}} \times 100 \]

**Phytotoxic Activity of *Cornus Macrophylla* Leaves:** Phytotoxicity assay was carried out for the crude methanolic extract and subsequent solvent dilution against *Lemna minor*. The medium was prepared and sterilized in autoclave at 121°C and 15 Psi for 15 min. Test samples (10 mg) of *Cornus macrophylla* leaves dissolved in methanol (1ml) served as stock solution. Nine flasks (Three for each dilution) were inoculated with 1000, 100 and 10 µl of the stock solution (500, 50 and 5ppm). The solvent was evaporated overnight under sterilized conditions and add 20 ml of the medium to each flask. Thereafter, 10 plants were added to each flask. One other flask, supplemented with solvent served as control and reference plant growth inhibitor (Paraquat), served as a standard phytotoxic drug. The flasks were plugged with cotton and placed in growth cabinet for 7 days. On the 7th day, the number of fronds per flask was counted. Results were analyzed as growth regulation in percentage, calculated with reference to the negative control.

**RESULTS**

**Phytochemicals:** The Phytochemical screening of crude methanolic extracts of *Cornus macrophylla* leaves revealed the presence of 07 different classes of phytochemicals, while glycosides and phlobutinins were not identified as shown in Table 1.

**Antioxidant Potential and Total Phenolic Contents:** The methanolic extracts of *C. macrophylla* leaves have shown a concentration dependent antioxidant potential in DPPH radical scavenging assay. Maximum (69.08%) activity was observed at 100 µg/ml, while 58.33, 50.44, 45.82 and 43.99% activity were observed at 80, 60, 40 and 20 µg/ml respectively, depicted in Table 2. The activity...
Table 1: Phytochemical screening of crude methanolic extracts of Cornus macrophylla leaves

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemicals</th>
<th>Reagents/Chemicals</th>
<th>Results</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Dragendorff’s</td>
<td>+</td>
<td>Orange red precipitation was found</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>+</td>
<td>Dark green colouration</td>
</tr>
<tr>
<td>3</td>
<td>Anthraquinones</td>
<td>HCl+CHCl3+NH3</td>
<td>+</td>
<td>Rose pink colour</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>Fehling's solution</td>
<td>-</td>
<td>Red precipitate was not observed</td>
</tr>
<tr>
<td>5</td>
<td>Reducing sugars</td>
<td>Fehling's solution</td>
<td>+</td>
<td>Orange red precipitation</td>
</tr>
<tr>
<td>6</td>
<td>Sapins</td>
<td>Distilled water</td>
<td>+</td>
<td>Frothing</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>NaOH + HCl</td>
<td>+</td>
<td>Discoloration</td>
</tr>
<tr>
<td>8</td>
<td>Phlobutaninns</td>
<td>HCl</td>
<td>-</td>
<td>Red precipitate not found</td>
</tr>
<tr>
<td>9</td>
<td>Terpenoids</td>
<td>CHCl3+H2SO4</td>
<td>+</td>
<td>Dark green colouration</td>
</tr>
</tbody>
</table>

Key + = Present - = Absent

Table 2: Comparison of %RSA of methanolic extract of Cornus macrophylla leaves (MECM) with Quercetin and Gallic acid

<table>
<thead>
<tr>
<th>Test Solutions</th>
<th>Concentration (µg/ml)</th>
<th>MECM</th>
<th>Quercetin</th>
<th>Gallic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample/Standards</td>
<td>20</td>
<td>40</td>
<td>60</td>
<td>80</td>
</tr>
<tr>
<td>MECM</td>
<td>43.9±1.4</td>
<td>45.8±1.6</td>
<td>50.4±0.9</td>
<td>58.3±2.1</td>
</tr>
<tr>
<td>Quercetin</td>
<td>95.3±1.5</td>
<td>96.3±1.1</td>
<td>97.1±0.6</td>
<td>98.0±0.8</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>85.4±1.3</td>
<td>92.4±1.1</td>
<td>95.1±0.7</td>
<td>96.2±0.8</td>
</tr>
</tbody>
</table>

Results are expressed as mean±SD, of three independent observations.

was compared with reference standard drugs, quercetin and gallic acid. Quercetin and gallic acid both displayed marked activity (95.35 and 85.49%) at lowest concentration (20 µg/ml). The IC₅₀ value determined for MECM was 57.87 µg/ml (Figure 1). Total Phenolic Contents (TPC) determined in the crude methanolic extract of Cornus macrophylla leaves was 97.3±2.06 mg of gallic acid equivalent per gram of dry weight of the plant material.

Brine Shrimps Lethality Bioassay: In cytotoxicity assay MECML displayed remarkable percent lethality against brine shrimp’s nauplii in a concentration dependent manner, as shown in Table 3. The test sample exhibited 11, 22, 36, 51, 57, 66 and 91% lethality at a dose of 10, 50, 100, 200, 250, 500 and 1000 µg/ml respectively with LC₅₀ 200 µg/ml. Etoposide was used as standard drug and showed profound cytotoxic effect (LC₅₀ < 0.1 µg/ml), depicted in Table 3.

Phytotoxic Potential: In the phytotoxic assay of the crude methanolic extract and subsequent dilutions of Cornus macrophylla leaves displayed a concentration dependent activity. The test sample at a concentration of 10 µg/ml has shown good activity (45%), excellent activity (65%) at 100 µg/ml, while profound activity (95%) was observed at 1000 µg/ml (Table 4). The reference standard drug Paraquat exhibited excellent activity at comparatively low dose (LD₅₀ 0.90 lg/ml), depicted in Table 4.
Table 3: Concentration dependent cytotoxic effect of CMMEL against Brine shrimp’s nauplii

<table>
<thead>
<tr>
<th>Concentration of extract (µg/ml)</th>
<th>No. of Brine shrimps Tested</th>
<th>No. of dead Brine shrimp</th>
<th>% Lethality</th>
<th>LC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>30</td>
<td>3.3±0.8</td>
<td>11.1</td>
<td>200</td>
</tr>
<tr>
<td>50</td>
<td>30</td>
<td>6.6±1.2</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>30</td>
<td>11.0±1.7</td>
<td>36.6</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>30</td>
<td>15.3±0.8</td>
<td>51.1</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>30</td>
<td>17±1.4</td>
<td>57.7</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>30</td>
<td>20±1.0</td>
<td>66.6</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>30</td>
<td>27.3±0.8</td>
<td>91.1</td>
<td></td>
</tr>
</tbody>
</table>

Data is represented as mean±SEM of the mean of three replicates of each dose (n=30). Etoposide LC$_{50}$ = 9.8 µg/ml.

Table 4: Concentration dependent phytotoxic potential of *Cornus macrophylla* leaves

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Conc. (µg/ml)</th>
<th>Test Samples</th>
<th>Control</th>
<th>% Growth Inhibition</th>
<th>LD$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemna minor</td>
<td>10</td>
<td>11±0.8</td>
<td>20</td>
<td>45±1.1</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>07±1.7</td>
<td></td>
<td>65±1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>01±1.8</td>
<td></td>
<td>95±0.8</td>
<td></td>
</tr>
</tbody>
</table>

Conc; concentration. Values are expressed as the mean±SD of three independent observations. Standard drug; Paraquat, LD$_{50}$ = 0.90 µg/ml.

**DISCUSSION**

The medicinal value of the plants is due to phytochemical constituents that cause definite pharmacological actions [17-19]. Plant produces a wide variety of secondary metabolites many of which have been reported to be of therapeutic value. Secondary metabolites comprise of alkaloids, flavonoids, saponins, tannin, phenolic compounds and many more [20]. Literature survey shows that out of 25000 species of higher plants in the world, only 5-10% has been chemically investigated. While a vast majority of the plant resource is waiting for discovery [21]. In the current study the methanolic extract of *C. macrophylla* leaves (MECM) was investigated for secondary metabolites. Tests for alkaloids, tannins, terpenoids, saponins, reducing sugar and anthraquinonnes were positive in MECML, while phlobutinins and glycosides were not detected in the methanolic extract of *C. macrophylla*, depicted in Table 1. Our results are in conformity to the results obtained from *Cornus controversa*, members of the cornaceae family, which have shown the presence of same classes of compounds including flavonoids, phenolic compounds and terpenoids [22]. The medicinal property of the plant could be attributed to phytochemicals residing in it.

Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) to decolorize in the presence of antioxidants. When DPPH accepts an electron (Donated by an antioxidant compound), the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. In DPPH radical scavenging assay the crude methanolic extract of *Cornus macrophylla* leaves exhibited marked antioxidant activity i.e 69.08% (100 µg/ml) with IC$_{50}$; 57.87 µg/ml, as shown in Table 2. Total Phenolic Contents (TPC) determined in the crude methanolic extract of *Cornus macrophylla* leaves was 97.3±2.06 mg of gallic acid equivalent per gram of dry weight of the plant material. Phenolic compounds are one of the largest groups of phytochemicals and have been tagged for most of the antioxidant activity of plants or plant products. The antioxidant activity of *C. macrophylla* might be due to their high phenolic contents.

Numerous researchers are focusing medicinal plants to discover novel, effective and safe compounds for the treatment of various challenging diseases like neoplasia [23]. A plethora of research studies are available which reflects the effectiveness and strong safety profile of various classes of phytochemicals. Saponins are group of secondary metabolites which possesses diversified pharmacological activities, like insecticidal [24], antioxidant [25] and anthelmintic [26]. Crude saponins extracted from *Teucrium stocksianum* possesses awesome cytotoxic effect (LC$_{50}$ <10 µg/ml) [27]. The cytotoxic effect of saponins has been previously reported by a number of researchers [28]. Human population is increasing at high rate which has a direct impact on the food resources. Numerous researchers are trying to elevate the per acre yield of the crops. Weeds are one of the major stumbling blocks in the crops production. Currently a number of effective synthetic weed killers are
available in the market but most of them are associated with serious hazardous effects. Therefore scientists are trying to discover plant based novel compounds for the eradication of weeds. A plethora of research is available which shows phytotoxic potential of medicinal plants [29]. In the current study the crude methanolic extract and subsequent dilutions of *Cornus macrophylla* leaves displayed profound phytotoxic activity (95% at 1000 µg/ml) against *Lemna minor* (Table 3). Thus it is concluded that the methanolic extract of *C. macrophylla* is a potential source of antioxidant and herbicidal constituents and need further scientific evaluation.

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**REFERENCES**


