Middle-East Journal of Scientific Research 9 (4): 516-519, 2011
ISSN 1990-9233


# Phytochemical Screening and Antimicrobial Activity of *Cornus macrophylla* Wall. ex Roxb

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**Abstract:** The tracking of phytoconstituents is important step which lead to the isolation of biologically active compounds. *Cornus macrophylla* is used as folk medicine for improving of improving liver and kidney functions, as tonic, analgesic and diuretic agents. Phytochemical screening of *Cornus macrophylla* revealed the presence of secondary metabolites such as alkaloids, steroids, terpenoids, flavonoids, reducing sugars and tannins. The ethyl acetate and methanolic and crude extracts showed the presence of alkaloids, terpenoids, tannins and reducing sugars while n-hexane, chloroform and ethyl acetate fractions revealed the existence of terpenoids, steroids, flavonoids, tannins and reducing sugars. The ethyl acetate crude and methanolic fractions were active against selected bacterial strains; displayed inhibitory zone of (14:0 mm) at the tested concentration (32 mg/ml). The medicinal values of *Cornus macrophylla* are due to the presence of detected metabolites.

**Key words:** Phytochemical screening · Metabolites · Antimicrobial activity

## INTRODUCTION

Genus *Cornus* belongs to family Cornaceae comprised of 55 species characterized by brilliant attractive colorful flowers and fruits, distributed in the temperate region. It is represented in Pakistan by three species, namely, *C. capitata, C. macrophylla, C. oblonga* [1,2]. Fruits of *Cornus* species are ingredients of many medical prescriptions in traditional medicines and have been successfully developed as a remedy for improving liver and kidney functions. Previously various classes of phytochemicals have been reported from the genus *Cornus,* which includes steroids [3], irridoids glycosides [4], anthocyanins [5], ellagic acid derivatives [6], phenolic compounds and flavonoids [7]. *Cornus* species are traditionally well known for their analgesic, tonic and diuretic activities and as a food preservative [8]. *In-vitro* studies indicated that fruits of *Cornus* species have anti-malarial, anti-allergic, anti-histamine, anti-microbial [9,10], anti-oxidant, anti-inflammatory [11], anti-diabetic [12], anti-cancer [13] and lipid peroxidative [14].

## MATERIALS AND METHODS

**Plant Material:** *Cornus macrophylla* whole plant was collected from Swat, Khyber Pakhtunkhwa Pakistan in the month of May 2008. The plant was identified by Mr. Mehboob Ali, Assistant Professor, Govt. Post Graduate Jehanzeb College Mingora, Swat, Pakistan. The voucher specimen (WA82) was deposited in the Herbarium of the Department of Botany, Islamia College University, Peshawar, Pakistan.

**Extraction and Fractionation:** Shade dried plant of *Cornus macrophylla* was soaked in methanol for 5 days. The extract was concentrated under vacuum at 40°C,

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using rotavapor, suspended in water and successively partitioned with n-hexane, chloroform and ethyl acetate and methanolic fractions.

**Micro-Organism Assortment and Preservation:** Four bacterial strains (*Proteus mirabilis*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*) were obtained from stock culture of Center of Biotechnology, University of Peshawar, Peshawar, Pakistan and stored in Muller-Hinton agar at low temperature (4°C) prior to subculture.

**Antimicrobial Assay of the Different Fractions Against Particular Bacterial Strains:** Modified agar well diffusion method was implemented to test the antibacterial activity of the fractions with the use of Müller-Hinton agar as medium. The cultures were prepared in triplicates incubated at 37°C temperature for a period of 24 to 72 hours. 0.6 mL of the broth culture of the test organism was put in a sterile Petri-dish and added 20 ml of the sterile molten MHA. Wells were jaded into the medium using 0.2 ml of the fractions using Streptomycin (2 mg/ml) as a standard of the antimicrobial agent. Inoculation was done for 1 h to ensure the diffusion of the antimicrobial agent into the medium. The inoculated plates were incubated for 24 h at 37°C. Diameters of the inhibition zone of microbial growth were measured in millimeters.

**Phytochemical Screening of Aerial Parts:** The chemical tests were performed on the hexane, chloroform, ethyl acetate and methanolic extracts of *Cornus macrophylla* using standard procedures [15-17] to recognize the ingredients.

**Alkaloids:** 0.2 g of each of the fractions was warmed with 2% $H_2SO_4$ for two minutes. The reaction mixtures were filtered and added a few drops of Dragendroff's reagent to each filtrate. Orange red precipitate indicates the presence of alkaloids moiety.

**Tannins:** A small quantity of each extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride were added to each of the filtrates. A dark green solution indicates the presence of tannins.

**Anthraquinones:** 0.5 g of each extract was boiled with 10 % HCl for few minutes on water bath. The reaction mixtures were filtered and allowed to cool. Equal volume of $CHCl_3$ was added to each filtrate. Few drops of 10% ammonia was added to each mixture and heated. Rose-pink colour formation indicates the presence of anthraquinones.

**Glycosides:** Each extract was hydrolyzed with HCl and neutralized with NaOH solution. A few drops of Fehling's solution A and B were added to each mixture. Formation of red precipitate indicates the presence of glycosides.

**Reducing Sugars:** Each extract was shaken with distilled water and filtered. The filtrates were boiled with few drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

**Saponins:** 0.2 g of each extract was shaken with 5ml of distilled water and heated to boiling. Frothing (appearance of creamy miss of small bubbles) shows the presence of saponins.

**Flavonoids:** 0.2 g of each extract was dissolved in diluted NaOH and few drops of HCl were added. A yellow solution that turns colourless indicates the presence of flavonoids.

**Phlobatanins:** 0.5g of each extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins.

**Steroids:** 2 ml of acetic anhydride was added to the mixture of 0.5 g of each extract and $H_2SO_4$ (2 ml). The colour change from violet to blue or green in some samples indicates the presence of steroids.

**Terpenoids:** 0.2 g of each extract was mixed with 2 ml of chloroform and concentrated $H_2SO_4$ (3ml) was carefully added to form a layer. The formation of a reddish brown coloration at the interface indicates positive results for the presence of terpenoids.

## RESULTS

The weight percentage yield of aerial parts and stem bark are shown in Table 1. The results of antimicrobial activity of various fractions and crude extract are shown in Table 2, while phytochemical screening of *Cornus macrophylla* is shown in Table 3.

Table 1: Weight and percentage yield of the crude extracts and fractions of *Cornus macrophylla*

| Solvent | Weight of crude extract | Percentage yield |
|---|---|---|
| n-Hexane extract (g) | 0.080 | 0.183 |
| Chloroform extract (g) | 1.5 | 3.44 |
| Ethyl acetate extract (g) | 0.85 | 1.949 |
| Methanol extract (g) | 1.153 | 2.644 |
| Crude extract (g) | 43.6 | 12.82 |

Table 2: Antibacterial assay of crude extract and fractions of *Cornus macrophylla*

| Sample | *P. m* | *S. a* | *E. c* | *B. c* |
|---|---|---|---|---|
| F1 | 12 | x | x | x |
| F2 | x | 11 | x | x |
| F3 | 12 | x | x | 13 |
| F4 | 8 | 10 | x | 14 |
| F5 | 10 | 11 | x | 8 |
| DMSO (Negative Control) | x | x | x | x |
| Imipenem 10µg/Disc (Positive Control) | 28 | 23 | 34 | 32 |

Concentration of stock solution 3mg/ml and 100 µL was used for assay

Keywords: well size= 6mm n-Hex.=F1, $CHCl_3$=F2, EtOAc=F3, MeOH=F4, Crude=F5.

*P. m= Proteus mirabilis, S.a = Staph aureus, E.c = Escherichia coli, B.c = Bacillus cereus*

Table 3: Phytochemical screening of n- hexane, chloroform, ethyl acetate, methanolic fractions and crude extract of *Cornus macrophylla*

| s/no | Chemical components | n-hex | $CHCl_3$ | EtOAc | MeOH | Crude |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | – | – | + | + | + |
| 2 | Terpenoids | + | + | + | + | + |
| 3 | Flavonoids | – | + | + | – | – |
| 4 | Anthraquinones | – | – | – | – | – |
| 5 | Tannins | – | – | + | + | + |
| 6 | Plobotanins | – | – | – | – | – |
| 7 | Saponins | – | – | – | – | – |
| 8 | Glycoside | – | – | – | – | – |
| 9 | Reducing sugars | – | + | + | + | + |
| 10 | Steroids | + | + | – | – | – |

Key: – = absent, + = present

## DISCUSSION

The result of the whole plant extract of *Cornus macrophylla* showed that ethyl acetate and methanolic fractions contain a greater proportion by mass of the component compounds. The medicinal value the plant can be correlated to the presence a variety of natural products present in it. The fractions taken from the crude extract of this plant are positive for terpenoids which showed different activities like antitumor and anticancer, Anti-inflammatory and antiviral/antibacterial [18] while ethyl acetate, methanolic fractions and crude extract were investigated for the presence of alkaloids which showed antibiotic activity [19].

The pharmacological activity of *C. maerophylla* was confirmed from the antimicrobial activity of various fractions. The ethyl acetate and methanolic fractions were active, showing activity against selected bacterial strains (Table 2) and thus displayed high inhibitory zone of (14:0 mm) at the tested concentration (32 mg/ml).

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