# Phytochemical Screening and Anthelmintic Activity of *Flueggea virosa*

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**Summary:** The present study involves the phytochemical screening and anthelmintic investigation of leaf and bark of plant *Flueggea virosa* (Roxb. ex Willd.) Voigt. The phytochemical analysis of leaf and bark of plant material showed the presence of reducing sugars, terpenoids, cardiac glycosides, flavonoids, saponins, anthraquinones and alkaloids. Anthelmintic activity of plant extract of *F. virosa* was carried out at four different concentrations 20, 50, 80, 100 mg/ml. Anthelmintic activity was evaluated in terms of time utilized for death and paralysis. All extracts showed significant anthelmintic potential which was dose dependent and compared to standard piperazine citrate.

**Key words:** *F. virosa*, Euphorbiaceae, Anthelmintic activity, Phytochemical screening.

## Introduction

According to world health organization (WHO) plants can provide variety of drugs for medicinal purposes [1]. Medicinal plants accumulate different organic compounds which are biologically active and used for the treatment of various human and animal ailments. The medicines obtained from plants are used in pharmaceutical and cosmetic industries [2]. The plants are medicinally important due to secondary metabolite such as alkaloids, saponins, flavonoids, cardiac glycosides, anthraqinones, terpenoids, and steroids [1]. Phytochemical are present in various parts of plants such as in leaves, fruits, roots, and stem etc. In wide range these phytochemicals are present in legumes, fruits, nuts, whole grains, spices, seeds, and vegetables [3].

Helminthosis is a disease caused by parasitic worms of the gastrointestinal tract. Helminthes infections are widespread in humans and animals, causing loss to livestock production and distressing human population [4]. These infections are highly prevalent in third world countries due to poor management practices [5]. Only a limited number of drugs are used to control helminthosis. Moreover, parasitic worms develop resistance over these drugs so there is need to develop new drugs from medicinal plants [6].

*Heamonchous contortus* is a pathogenic parasite which is responsible for heamonchosis. This disease is attributed to anemia due to blood loss by blood sucking of parasitic worms [7].

Traditionally practitioners are using plants as medicines for treating helminthic infections, but scientific validation of these drugs is still not available [5]. Phytochemicals present in the plants are considered to be responsible for the anthelmintic activity [6]. A large number of plants can be investigated on the basis of their traditional use by the local people as aboriginal people attain the knowledge of plants from their ancestors [8].

The plant *Flueggea virosa* belongs to family euphorbiaceae. *Euphorbia* species have anti-inflammatory, analgesics, heamostatic and wound healing properties [9]. This plant belongs to genus *Flueggea*, members of this genus have analgesic and antiproliferative properties. This plant naturally found in tropical Africa, Asia and Japan [10]. On the basis of ethnomedicinl value of family euphorbiaceae the present study was carried out.

## Experimental

### *Plant Material*

Plant specimen *Flueggea virosa* (Roxb. ex Willd.) Voigt obtained from District Kotli, Azad Jammu & Kashmir (AJK). The plant was identified with voucher no. GC. Herb. Bot. 2947 and submitted to the Dr. Sultan Ahmed herbarium, Department of

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*Maceration of the plant material*

The shade dried plant material (500 mg) was crushed, powdered and soaked in 1 L solvents including petroleum ether, chloroform, methanol and distilled water for 15 days. Resulting extracts were filtered and concentrated under rotary evaporator.

*Phytochemical screening*

Qualitative phytochemical analysis of bark and leaf extracts of *F. virosa* was carried by using specific methods. Alkaloid detection was carried out by Sethi [11] and Wagner method [12], terpenoids detection by Harborne method [13], flavonoids by Bello [14] and Mace strategy [15], tannins by Evans method [16], saponins by Akinjogunla methodology [17], reducing sugars by Fehling method [18], cardiac glycosides by Onwukaeme strategy [19] and anthraquinones by Evans method [16].

*Anthelmintic activity*

*Test organisms*

For the anthelmintic activity of the plant *Haemonchus contortus* parasite was used which is obtained from the abomasum of slaughtered goat. 0.9 % NaCl was used to wash the abomasum. The test organisms were placed in solution of 0.9 % NaCl until further examination.

*Methodology*

*Preparation of solutions*

Solutions were freshly prepared before the beginning of the anthelmintic activity.

*0.9 % NaCl solution*

0.9 g of NaCl was dissolved in 100.0 mL distilled water. The resulting solution was autoclaved for 15 minutes at 121°C and 15 psi pressure. This solution was utilized to adjust the consistency of microbial cell in inoculum.

*10 mg/mL piperazine citrate*

In distilled water 0.2 g of piperazine citrate ($3C_4H_{10}N_2.2C_6H_8O_7$) was dissolved and raises the final volume up to 20.0 mL by addition distilled water.

*Preparation of plant dilutions*

Dilutions of root and bark extracts of plant were prepared in four different concentrations (20, 50, 80, 100 mg/mL).

*Procedure*

The anthelmintic activity was performed according to the method used by Ajaiyeoba *et al.* [20]. *Heamonchous contortus* was placed in petri dishes containing four different concentrations (20, 50, 80 and 100 mg/mL) of each extract of *F. virosa*. Each petri plate was assigned with 5 same sized worms and observed for paralysis and death. The time for paralysis and death was recorded in minutes. In the same way, in one petri-plate 10.0 mL of piperazine citrate for standard and in other petri plate saline solution for control was added. All plates were placed for 4 hours at room temperature.

*Statistical analysis*

Statistical analysis of results was carried out and the procedure was run in triplicates [21, 22].

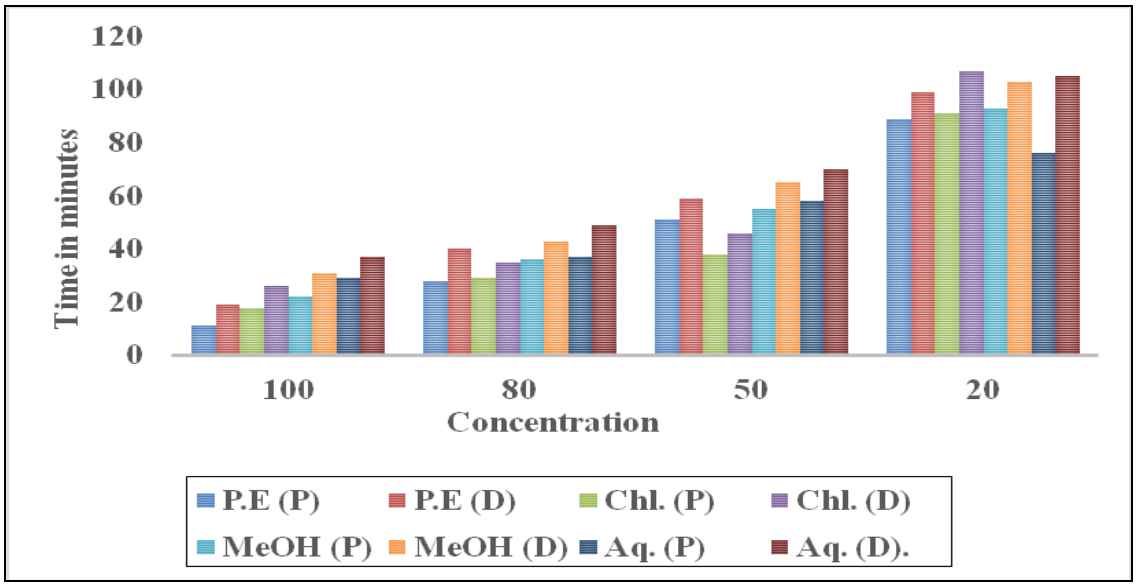
## Results and Discussion

The qualitative phytochemical analysis of leaf and bark extracts of *Flueggea virosa* was performed to detect the presence of alkaloids, cardiac glycosides, flavonoids, anthraquinones, tannins, reducing sugars, saponins and terpenoids. The reducing sugars, terpenoids, tannins and saponins found in all the extracts of leaf and bark. Flavonoids are present in chloroform, methanol and aqueous extracts of leaf and bark. Alkaloid was present in methanol and distilled water extracts of leaf and bark. The cardiac glycosides present only in chloroform extract of leaf and bark. Anthraquinones were present only in methanolic extracts of leaf and bark (Table-1).

Table-1: Phytochemical analysis of the leaf extracts of *F. virosa*

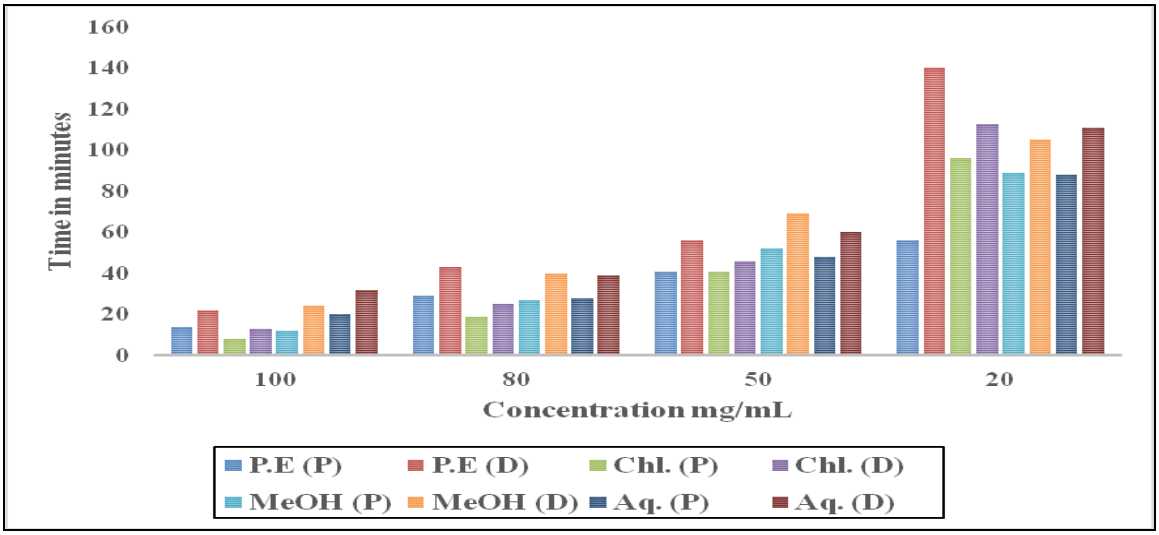
| Plant material | | Phytochemicals | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Plant parts | Extracts | Alkaloids | Flavonoids | Terpenoids | Saponins | Tannins | Anthraquinones | Reducing sugar | Cardiac glycosides |
| Leaves | Petroleum ether | - | + | + | + | + | - | + | - |
| | Chloroform | - | - | + | + | + | - | + | + |
| | Methanol | + | + | + | + | + | + | + | - |
| | Aqueous | + | + | + | + | + | - | + | - |
| Bark | Petroleum ether | - | - | + | + | + | - | _ | + |
| | Chloroform | - | + | + | + | + | + | + | + |
| | Methanol | + | + | + | + | + | - | + | - |
| | Aqueous | - | + | + | + | + | - | + | - |

*Phytochemical detection key: − = Absent, + = Present




*(P) = Paralysis, (D) = Death. *P.E = Petroleum ether, Chl.= Chloroform, MeOH = Methanol, Aq. = Aqueous.

Fig. 1: Anthelmintic activity of leaf extracts of *F. virosa.*




*(P) = Paralysis, (D) = Death.*P.E = Petroleum ether, Chl. = Chloroform, MeOH = Methanol, Aq. = Aqueous.

Fig. 2: Anthelmintic activity of bark extract of *F. virosa.*

The anthelmintic activity of the plant extracts of *F. virosa* was carried out at different concentrations (20, 50, 80, 100 mg/mL) of each extract. Time taken for death and paralysis was recorded in minutes. The minimum time duration of paralysis and death of leaf extracts of *F. virosa* was occupied at concentration of 100 mg/mL while the maximum time was observed at concentration of 20 mg/mL. The time for paralysis ranged from 11-29 minutes at concentration of 100 mg/mL. The maximum time consumed by aqueous extract and minimum time was consumed by petroleum ether extract. The time for death at concentration of 100 mg/ mL was ranged from 19-37 minutes. The extracts of non-polar solvents were more efficient in causing the death and paralysis of worms (Table-2, Fig.2).

Table-2: Anthelmintic activity of leaf extracts of *Flueggea virosa*

| Solvents | Concentration (mg/mL) | Time taken for paralysis (P) in minutes | Time taken for death (D) in minutes |
|---|---|---|---|
| **Petroleum ether** | **100** | **11 ± 0.2** | **19 ± 0.1** |
| | **80** | **28 ± 0.6** | **40 ± 1.5** |
| | **50** | **51 ± 0.1** | **59 ± 0.3** |
| | **20** | **89 ± 0.5** | **99 ± 1.2** |
| **Chloroform** | **100** | **18 ± 0.3** | **26 ± 0.4** |
| | **80** | **29 ± 0.2** | **35 ± 0.3** |
| | **50** | **38 ± 0.4** | **46 ± 1.5** |
| | **20** | **91 ± 0.5** | **107 ± 0.1** |
| **Methanol** | **100** | **22 ± 0.2** | **31 ± 0.5** |
| | **80** | **36 ± 1.1** | **43 ± 0.5** |
| | **50** | **55 ± 0.1** | **65 ± 0.4** |
| | **20** | **93 ± 0.5** | **103 ± 0.9** |
| **Aqueous** | **100** | **29 ± 0.1** | **37 ± 0.6** |
| | **80** | **37 ± 0.5** | **49 ± 1.5** |
| | **50** | **58 ± 1.0** | **70 ± 1.0** |
| | **20** | **76 ± 0.5** | **105 ± 0.7** |

The minimum time for paralysis and death was observed at concentration of 100 mg/mL of bark extracts of *F. virosa* while the maximum time occupied at concentration of 20 mg/mL. The time for paralysis ranged from 8-20 minutes and for death 13-32 minutes at concentration of 100 mg/mL (Table-3). The maximum time was utilized by the aqueous extracts and minimum time consumed by chloroform extract. All the extracts of bark of *F. virosa* were effective in causing the paralysis and eventually death of worms.

Table-3: Anthelmintic activity of bark extract of *F. virosa*.

| Solvents | Concentration (mg/mL) | Time taken for paralysis (P) in minutes | Time taken for death (D) in minutes |
|---|---|---|---|
| **Petroleum ether** | **100** | 14 ± 0.1 | 22 ± 0.1 |
| | **80** | 29 ± 0.4 | 43 ± 1.4 |
| | **50** | 41 ± 0.3 | 56 ± 0.5 |
| | **20** | 56 ± 0.5 | 140 ± 1.5 |
| **Chloroform** | **100** | 08 ± 0.2 | 13 ± 0.6 |
| | **80** | 19 ± 0.1 | 25 ± 0.7 |
| | **50** | 41 ± 0.4 | 46 ± 1.3 |
| | **20** | 96 ± 0.8 | 113 ± 1.1 |
| **Methanol** | **100** | 12 ± 0.1 | 24 ± 0.5 |
| | **80** | 27 ± 1.9 | 40 ± 0.1 |
| | **50** | 52 ± 1.1 | 69 ± 0.7 |
| | **20** | 89 ± 0.7 | 105 ± 1.0 |
| **Aqueous** | **100** | 20 ± 0.3 | 32 ± 0.1 |
| | **80** | 28 ± 0.6 | 39 ±1.1 |
| | **50** | 48 ± 1.0 | 60 ± 0.1 |
| | **20** | 88 ± 0.5 | 111 ± 0.5 |

The results of anthelmintic activity showed that, the activity was dose dependent which was in accordance to Kosalge, Vidhayar, Ahmed and partap [4, 5, 23, 24]. The inverse relation was found between the time utilized for death or paralysis and amount of the extracts utilized [25]. It could be assumed that anthelmintic potential of different extracts of plants may be due to the secondary metabolites present in plant of *F. virosa*.

## Conclusion

The qualitative phytochemical analysis of plant material *F. virosa* showed the presence of reducing sugars, terpenoids, cardiac glycosides, flavonoids, saponins, anthraquinones and alkaloids. Both leaf and bark extracts of plants showed significant anthelmintic potential. Further research work is needed to isolate and identify more chemical constituents responsible for anthelmintic activity.

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