

***In vitro* screening of *Cymbopogon jwarancusa* and *Conyza canadensis* against liver flukes**

Andeela Shafiq¹, Rabia Kanwal², Rahamat Ullah Qureshi³ and Farhana Riaz Chaudhry^{2*}

¹Department of Biology, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi 4600, Pakistan

²Department of Zoology, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi 4600, Pakistan

³Department of Botany, Pir Mehr Ali Shah-Arid Agriculture University, Rawalpindi 4600, Pakistan

*Corresponding author email: farhana_ahmer@yahoo.com

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Abstract. Aim of present study was to screen medicinal plants for flukicidal activity *in vitro* to develop alternative sources of treatment for trematodes infection. For this purpose, crude methanolic extracts (CME) of *Cymbopogon jwarancusa* and *Conyza canadensis* were prepared and live adult flukes viz; *Fasciola gigantica*, and *Paramphistomum cervi* isolated from liver and bile ducts of slaughtered buffalo were subjected to different drug concentrations as well as positive and negative control. Motility inhibition and paralysis leading to the death of parasites was considered as flukicidal activity of plants extracts. The results revealed that CME of *C. jwarancusa* and *C. canadensis* showed significant ($P < 0.05$) flukicidal activity compared to positive control. Also there was a significant effect of different concentrations ($P < 0.05$) and exposure of time on the flukes ($P < 0.05$). Furthermore, ED₅₀ for *C. jwarancusa* and *C. canadensis* against *F. gigantica* were 13.1 and 41.4 mg/ml, respectively. In the case of *P. cervi*, it was 10.8 and 29.0 mg/ml. It can be concluded that both tested plants showed greater flukicidal activity as compared to positive control with Albendazole till the 8th hr. These potent plants need further studies *in vivo* to elucidate their mode of action.

INTRODUCTION

Amongst the helminth, *Fasciola gigantica* and *Paramphistomum cervi* are two major trematode parasites in the liver of ruminants, affecting farmers economically by reducing weight gain and milk production around the world, mainly reported from developing countries (Mas-Coma *et al.*, 2007). Flukes cause extensive damage to liver parenchyma, haemorrhage, anemia, hypoalbuminaemia, edema and eosinophilia in ruminants (Talukder *et al.*, 2010). Livestock owners can derive substantial benefits with the use of synthetic anthelmintic in controlling livestock trematodosis (Gaasenbeek *et al.*, 2001). It is an established fact that indiscriminate and injudicious use of synthetic drugs has led to the development of resistant populations of flukes in ruminants (Fairweather, 2009). The emergence of

resistance has prompted researchers to seek alternative drugs which are sustainable and environmentally acceptable.

In this regard, herbal medicine may be the best option as plants have been used in conventional medicine. Plants are important nutraceuticals as they have both nutritional and pharmaceutical value which acts in polyvalent way, showing better clinical effects and having greater depth and breadth than those seen in drug therapy ((Mehlhorn *et al.*, 2010). A survey of 2675 veterinarians in Australia, Germany and Switzerland suggests that about three-fourth of them were using herbal medicines to treat chronic diseases and as adjunct therapy (Hahn *et al.*, 2005).

Globally, various studies have scientifically validated herbal drugs for their flukicidal activity viz; in Sudan (Koko, 2006), Thailand (Saowakon *et al.*, 2009), USA

(Ferreira *et al.*, 2011), Brazil (Godinho *et al.*, 2014), India (Kumar and Singh, 2014). Currently no work has been reported from Pakistan regarding flukicidal potential of medicinal plants.

As Pakistan is blessed with wide range of flora consisting of many medicinal plants, the present study was designed to investigate *in vitro* parasitic activity of *Cymbopogon jwarancusa* and *Conyza canadensis* plants in Pothwar region-Pakistan against liver flukes. Selected plant species have several medicinal and pesticidal properties such as antioxidant, antiviral, antibacterial, antifungal. These plants were not tested previously for any scientific evidence for the flukicidal potential. These *in vitro* trials may act as a base for evaluation of the effectiveness of the studied plant material *in vivo* against devastating flukes.

MATERIALS AND METHODS

Collection of Plants

Two medicinal plants namely, *Cymbopogon jwarancusa* L. (Poaceae) voucher no. 2356 (common name, khawi) whole plant, and *Conyza canadensis* L (Asteraceae) voucher no. 2378, (common name, chitti buti) aerial part were selected for *in vitro* evaluation of flukicidal activity. Selection of these plants was based on their local EVM uses by farmers/herders (Raziq *et al.*, 2010). The plant materials were collected from the growing locality Pothwar and identified by using the taxonomic key (Cope *et al.*, 1982; Kayani *et al.*, 2007).

Preparation of methanolic extracts

Selected plant materials dried, cleaned, pulverized and stored in sealed bags. It was then soaked in absolute methanol in 1:10 (w/v) at room temperature (25-30°C). Soaked materials were incubated in shaker incubator for 3 days at 25°C. The filtrates were collected through a piece of Whatt man No. 1 filter paper and the plant materials were re-soaked twice. The combined filtrate was evaporated at 40°C to yield a thick and dark colored crude extract. This extract was stored at 4°C until use.

Collection of parasites

Liver flukes viz., *F. gigantica*, and *P. cervi* were extracted from the liver and bile duct of freshly slaughtered buffaloes, in Sihala slaughter house, Rawalpindi.

Evaluation of flukicidal activity of extracts

Flukicidal assay was carried as described by Tendon *et al.* (1997) with some modifications. The adult flukes were incubated at 30-37°C in positive control (Albendazole), in negative control (PBS) and crude methanolic extracts of plants with different dose levels of 0.75, 1.5, 3, 6, 12.5, 25, 50, 100 mg/ml. Each concentration contained three replicates, wherein each replicate contained flukes (*F. gigantica* n=4; *P. cervi* n=6) with total (*F. gigantica* n=12; *P. cervi* n=18). The fluke motility was observed at regular interval after each hr till 8 hrs. Post treatment mortality of flukes was confirmed by checking the revival of motility in PBS. When the experiment was terminated, number of dead and alive worms was counted at each dose level.

Statistical analysis

The effective dose (ED50) concentration for each extract was determined by Probit analysis. Significance between means was determined by the ANOVA and DMRT in MSTAT-C version 2.10.

RESULTS

It was observed that crude methanolic extracts (CME) of *C. jwarancusa* and *C. canadensis* plants had significant (P<0.05) flukicidal activity against tested flukes (*Fasciola gigantica* and *Paramphistome cervi*) in time and dose dependant manner as compared to positive control (Albendazole)

Evaluation of fasciolicidal potential

All the tested concentrations 0.75, 1.5, 3, 6, 12.5, 25, 50 and 100 mg/ml of both plants exhibited significant parasitic activity (P<0.05) against *F. gigantica*. Both the plants showed 100% efficiency at 12.5, 25, 50 and 100 mg/ml concentrations till 8 hrs as compared to synthetic drug. Moreover *C.*

jwarancusa achieved the best parasitic activity even at the lower concentrations of 6 and 3mg/ml against *Fasciola* as with same concentrations of *C. canadensis*. Hence *C. jwarancusa* displayed higher fasciolicidal potential with high efficacy (88.5%) and with a reduction of ED50 (13.1 mg/ml) as compare to *C. canadensis* showing the lesser amount of efficacy (65.6%) with larger ED50 (41.4 mg/ml), respectively. *In vitro* screening, the activities of both plants were found to be time and concentration dependent as shown in Figure 1 and 3.

Evaluation of paramphistomocidal potential

Data revealed that *C. jwarancusa* exhibited significantly ($P < 0.05$) higher paramphistomocidal activity (ED50=10.8 mg/ml; efficacy = 90.2%) as compared to *C. canadensis* with efficacy=72.9% and ED50=29.0 mg/ml. The CME of *C. canadensis* were found completely inactive against *P. cervi* at the doses of 0.75, 1.5, 3 and 6 mg/ml as compared to the *C. jwarancusa* at the same doses.

Tested plants displayed concentration and time dependent paramphistomocidal activity as shown in Figure 2 and 4.

Response of flukes to Positive control Albaendazole

Both flukes were exposed to positive control at 10mg/ml for 8 hrs no mortality was recorded till the end of experiment and hence found to be resistant and completely ineffective ($P < 0.05$) as compared to selected medicinal plants extracts.

DISCUSSION

This is the first primary report on *in vitro* trematocidal properties of crude methanolic extract (CME) of *C. jwarancusa* and *C. Canadensis* against *F. gigantica* and *P. cervi*. The result of the present study clearly demonstrated that CME of both plants are potent source of flukicide against *F. gigantica* and *P. cervi* in time and dose dependent manner as compared to synthetic

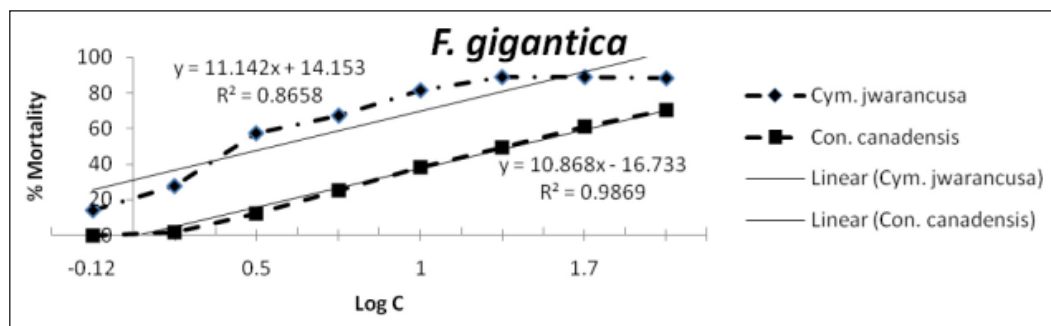


Figure 1. *In vitro* anthelmintic activities of crude methanolic extracts of tested plants in relation to percent mortality and dose/concentration (5-40 mg/ml) against *F. gigantica*

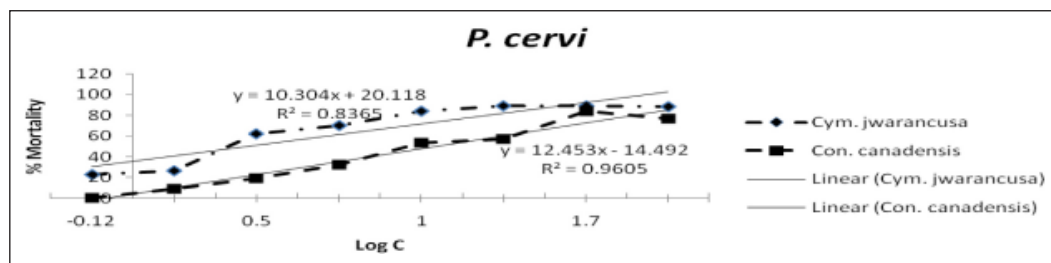


Figure 2. *In vitro* anthelmintic activities of crude methanolic extracts of tested plants in relation to percent mortality and dose/concentration (5-40 mg/ml) against *P. cervi*

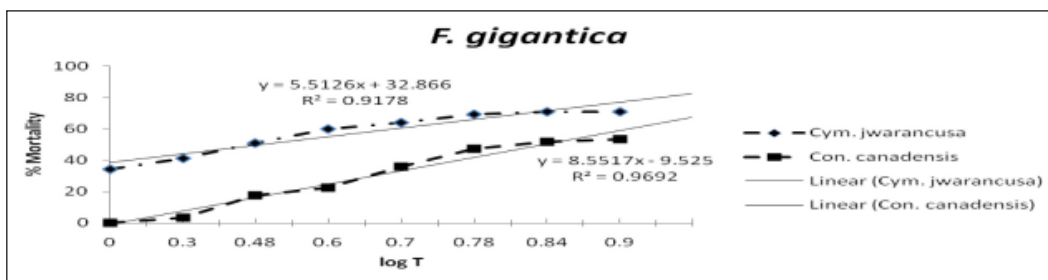


Figure 3. *In vitro* anthelmintic activities of crude methanolic extracts of tested plants in relation to time (0 hr – 8 hr of post treatment) and percent mortality against *F. gigantica*

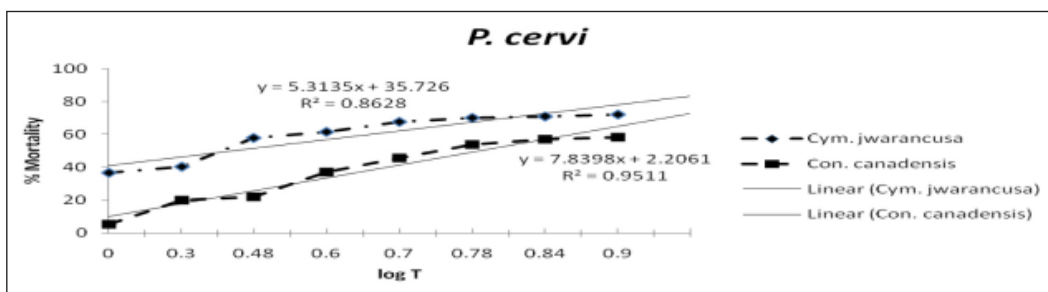


Figure 4. *In vitro* anthelmintic activities of crude methanolic extracts of tested plants in relation to time (0 hr – 8 hr of post treatment) and percent mortality against *P. cervi*

drug. This dependency may be due to the uptake of the active moiety which progressively increases in fluke body with increase in exposure period.

The promising flukicidal activity of *C. jwarancusa* may be due to the presence of chemical constituents like aromatic alkaloids and terpenes. Kayani *et al.* (2007) & Ahmed *et al.* (2010) documented aromatic alkaloids to be good anthelmintic compounds as it may be acting on central nervous system and have capacity to intercalate with fluke DNA which ultimately caused paralysis and mortality of the flukes. Terpenes are involved in tegumental membrane disintegration due to their lipophilic compounds which possibly may result in disturbing the normal bodily biochemical and physiological processes. The antifungal, antibacterial, anti-filarial, anti-ameobic and larvicidal activity of *C. jwarancusa* extract were reported by Shah *et al.* (2011) yet there was no report on the flukicidal activity against *F. gigantica* and *P. cervi*.

Conyza canadensis exhibited flukicidal activity but it was found to show less activity than *C. jwarancusa*. Our result are in accordance with the previous studies Shaukat *et al.* (2004) which showed that aqueous extracts of the *Conyza canadensis* *in vitro* having good nematocidal effect against plant-parasitic nematodes *Xiphinema americanum*, *Tylenchulus semipenetrans* and *Meloidogyne incognita*. Thus anthelmintic activity may be attributed due to the presence of certain previously reported active component such as C10 acetylenes, sesquiterpene hydrocarbons, phenolic compounds flavonoids and sterols, triterpenes, and sphingolipids (Xie *et al.*, 2007). Said components might be the potential to change the different enzyme activity in fluke body cause paralytic effect. Although the antimicrobial, antifungal and antiviral activity of *Conyza canadensis* extract were reported by various workers (Edziri *et al.*, 2011; Veres *et al.*, 2012) yet there was no report on the anthelmintic activity of this plant against *F. gigantica*.

Within entire series of trial, no fluke death was recorded in positive control till 8 hr, showing the ineffectiveness of tested chemical drug which might be due to the development of resistance within flukes. In accordance with those previous finding by Moll *et al.* (2000); Alvarez-Sanchez *et al.* (2006) and Cruz *et al.* (2010) illustrated resistance to triclabendazole (TCBZ) drug which has been emerging in various locations worldwide against flukes in ruminants. Infact TCBZ a benzimidazole-derivatives exert their anthelmintic activity by binding to β -tubulin, which interferes with the polymerisation of the microtubuli. Several authors like Robinson *et al.* (2002); Fairweather (2005); Lousie *et al.* (2008) and others show that there is an extensive polymorphism of the β -tubulin gene in susceptible parasite populations leading to depolymerisation of microtubules and instability in the cytoskeleton.

In vitro screening systems are valuable and fundamental step to discover potential anthelmintic candidates that can be employed for *in vivo* tests. Thus it can be concluded from the above study that methanolic extracts of *C. jwarancusa* and *C. canadensis* may be used as potent flukicide. For proper utilization of these plant products as flukicide further *in vivo* studied are however, necessary to elucidate the mode of action in fluke bodies.

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