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In vitro anthelmintic activity of *Physalis minima* ethanolic leaves and stem extracts against *Paramphistomum cervi* from cattle

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Abstract

Background: Crude ethanolic extracts of leaves and stem of *Physalis minima* (Solanaceae) were evaluated for in vitro anthelmintic activity on the Bangladeshi mature parasitic flatworm *Paramphistomum cervi (*Trematoda*)* in cattle. To compare the test results, Albendazole was used as a standard drug.

Methods: A leaves and stem extract of the *P. minima* was prepared in a Soxhlet apparatus using ethanol as a solvent. After concentrating in a vacuum rotary evaporator, the extract was analyzed for phytochemical activities. In vitro anthelmintic activity was tested against the flat worm *P. cervi*.

Results: The results of anthelmintic activity of *P. minima* were evaluated by paralysis instead of taking as of the paralysis time and death time of the flatworm. The study concluded that the ethanolic leaves and stem extracts of *P. minima* had anthelmintic activity in a dose-dependent inhibition of spontaneous motility of flatworm. From the screening experiment, the crude extracts showed the best anthelmintic activity. Furthermore, our phytochemical studies point out that ethanolic extract of the leaves and stem of *P. minima* contains flavonoids, phenols alkaloids, terpenoids, tannins, steroids, proteins and cardiac glycosides.

Conclusions: The studies of total phenolic and flavonoids content were quantified for all parts of the plant. The results of the present study suggest that *P. minima* extracts are a promising alternative to the commercially available anthelmintics for the treatment of gastrointestinal nematodes of cattle. Further research is required to confirm the possibility of the antimicrobial and antiproliferative applications.

Keywords: *Physalis minima*, Anthelmintics, *P. cervi*, Parasitic worm, Medicinal herbs, Phytochemical

Background

The regular utilization of medicinal herbs in developing countries, and in vitro and in vivo studies have been performed to explore the plant species' usefulness as another anthelmintics activity (Assis et al. 2003; Pereira et al. 2009). Recently plant-based medicines use is increasing day by day, that is why pharmaceutical industries are also showing their interest in formulation with natural products (Prasad et al. 2012). *Physalis minima* (Family: Solanaceae) is a perennial medicinal plant. In its maturity, this tropical annual herbs height is 20–50 cm. The plant is distributed in the wastelands and roadsides in India, Bangladesh, Afghanistan, Baluchistan, tropical Africa and Australia (Supriya et al. 2016).The plant *P. minima* have a remarkable effect in traditional medicine as an appetizing, tonic, laxative, diuretic. It has also valuable anti-inflammatory, antigonorrheic, enlargement of the spleen and abdominal disturbances. The leaf juice when mixed with water and mustard oils is used for the treatment of earache. Again decoction of the leaf, root and fruit was used together with tea for the treatment of high blood pressure, diabetes and malaria (Fasihuddin and

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Ghazally 2003; Karpagasundari and Kulothungan, 2014). Some of these medicinal values were scientifically documented (Jyothibasu et al. 2012; Patel et al. 2011). Phytochemical screening was carried out by testing of different chemical groups present in *P.minima* fruits extract (Sunitha et al. 2018). *Physalis minima* leaf extracts showed antimicrobial activity against selected pathogenic fungi and bacteria, following broth dilution assay (Shariff et al. 2006). Helminth infections are frequently found in the community. Besides, it has been documented as reason of much sensitivity and the preference to manage these pathologies could be one of the uses of herbs of cattle. The literature review reveals that *P. minima* are applied to cure different types of gastrointestinal problems. Various kinds of plant extracts like *Annona muricata* aqueous leaf extract have been showing anthelmintic effects and also phytochemical studies which specify the presence of phenolic compounds (Ferreira et al. 2013).

The aim of the current study was therefore to assess the in vitro anthelmintic potential of the leaves and stem extract of *P. minima* on nematode parasite *Paramphistomum cervi* from cattle. In this manuscript, first time we are reporting the anthelmintic activity of the leaves and stem of medicinal plant *P. minima* with their phytochemical studies.

Methods

Collection and identification of the plant

The *P. minima* plant was collected from the medicinal plant garden Khwaja Yunus Ali University, Enayetpur, Sirajgonj, Bangladesh. The plant was identified and verified by Professor Dr. AHM Mahbubur Rahman (Taxonomist), Department of Botany, University of Rajshahi, Bangladesh. The voucher specimen (no. 317) was preserved and deposited in the herbarium of the Department of Botany, University of Rajshahi, Bangladesh.

The leaves and steam of *P. minima* were sun-dried for one week and leaves and stem were pulverized in a mortar and pastel to produce fine powder materials. The powder materials were screened though a 50 mesh screen and stored for experimental purposes.

Preparation of the plant extract

The air-dried fine powder of the leaves and stem of *P. minima* were homogenized with ethanol using soxhlet's apparatus. The 200 gm powdered leaves and stem were defatted with petroleum ether (60–80 °C) and then subjected separately in a soxhlet extraction apparatus with ethanol (1L) for extraction. The ethanolic extract was collected and concentrated at 30 °C under reduced pressure in a rotary evaporator. 0.125, 0.25, 0.50 and 1gm of extracts were taken in four separate test tubes and mixed with 0.2% v/v of Tween 80 which was used as a suspending agent. The suspension of ethanol extract of *P. minima* at the concentrations of 12.5, 25, 50 and 100 mg/ml was prepared. After that, the final volume was adjusted to 10 ml for each concentration through phosphate-buffered saline.

Phytochemical analysis

Phytochemical analysis was performed using standard procedures available in the literature (Trease and Evans 1989; Govind and Madhuri 2006). Phytochemical screening was carried out by testing of different chemical groups present in *P.minima* fruits extract (Sunitha et al. 2018). Presently our research focused on the presence of chemical groups individually in leaves and stem extract of this plant for detection of alkaloids, steroids, tannins, flavonoids, glycosides, reducing sugar, saponins, terpenoids, quinine, phenols, proteins and gum in *P. minima* extract.

Test for reducing sugars (Fehling's test)

Two milliliters of plant extract was added with 1 ml of a mixture of equal volumes of Fehling's solutions A and B and boiled for some minutes for detection of reducing sugars. No precipitate was found which indicates the absence of reducing sugar (Ramakrishna et al. 1994).

Tests for tannins

Five milliliters of plant extract was taken in a test tube and added 1 ml of 5% ferric chloride solution for the detection of tannins. The black color appeared that identify the presence of tannins (Mace 1963).

Test for flavonoids

Each 2 milliliters of leaves and stem extracts was taken in a test tube and added a small amount of dilute NaOH, yellow colour has appeared. Now, a few drops of 70% dilute HCl was added and yellow colour was disappeared. This point out the presence of flavonoids in the leaves and stem extracts (Evans 1970).

Test for saponin

One milliliter of extract was diluted with 20 ml of distilled water and shaken in a measured-off test tube for 10–15 min. Foam layer has not appeared which specifies the lack of saponin properties (Kokate 1999).

Test for steroids

One milliliter of chloroform extract solution was taken and then added 2 ml Libermann-Burchard reagent. A greenish color appeared that specifies the presence of steroids (Finar 1986).

Tests for alkaloids

Two milliliters of each extract was taken in test tubes and added 0.2 ml of dilute HCl. In these two portions of the solution, 1 ml of Mayer's reagent was added to one portion and 1 ml of Dragendorff's reagent was added to the other. The formation of a yellow color precipitate (with Mayer's reagent) or orange brown precipitate (with Dragendorff's reagent) was regarded as positive for the presence of alkaloids (Evans 1970; Waldi 1965).

Tests for proteins

Take from each extract (2 ml) and add 1 ml of 40% caustic soda and a few drops of 1% copper sulfate. The formation of violet color indicates the presence of peptide bond molecules within the sample extract (Gahan 1984).

Tests for cardiac glycosides (Keller-Killiani test)

Take from every extract (1 ml) and add 0.5 ml of glacial acetic acid and 1–2 drops of 1% aqueous ferric chloride solution. The creation of a brown ring at the edge confirms the existence of cardiac glycosides in the solution of extract (Mace 1963).

Tests for terpenoids

Take from each extract (1 ml) and add 0.5 ml of chloroform and a few drops of concentrated sulphuric acid, reddish-brown precipitate to confirm the presence of terpenoids in the extract (Mace 1963).

Test for quinone

One milliliter of extract was taken and 1 ml of conc. H_2SO_4 was added. No color was formed which indicates the absence of quinone (Peach and Tracoy 1955).

Test for gums

Five milliliters from the extract was taken and then Molish reagent and sulphuric acid were added with them. A red or reddish-violet ring was formed at the junction of the two layers representing the presence of gums (Whistler and BeMiller 1993).

Anthelmintic activity tests

Collection of parasite samples

Collection *Paramphistomum cervi* from freshly slaughtered cattle were supplied from slaughter houses and confirmed by experts. After cleaning, parasites were stored in 0.9% of PBS of pH 7.4 at 37 ± 1 °C.

Standard drug used

Albendazole (collected from Square Pharmaceuticals Ltd., Bangladesh) suspension (concentration of 50 mg/ml) was used as the standard anthelmintic drug.

In vitro tests

For the present study, live parasites *P. cervi* of cattle were selected randomly (Dash et al. 2002; Szewezuk et al. 2003; Shivkar and Kumar 2008). Before experimentation, the flatworms were kept in the laboratory environment. The flatworms were divided into four groups and of three flatworms for each one. Albendazole suspension was used as a standard drug at a concentration 50 mg/ml, and it poured into a petri dish. The sample extracts were prepared in different concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml and 100 mg/ml). Control group was treated with 0.1% tween-80 in Phosphate-buffered saline. Three parasites nearly equal in size were placed in each petri dishes and observed at room temperature (Kaushik et al. 1974). The time taken for complete paralysis and death as compared to control were recorded (Williams et al. 2014; Islam et al. 2015). The paralysis time and death time were examined according to every sample. That time was mentioned as a paralysis time when the worms to became motionless. For ensuring the death external stimuli were applied continuously to the each worm which indicates that if alive the worm, its movement would be encourage (Lal et al. 1976).

Statistical analysis

All values are reported as mean ± S.E.M. The statistical differences among groups were assessed using the Duncan multiple range test and analysis of variance (ANOVA). A value of $P < 0.05$ was considered significant. Statistical analysis was performed using the SAS for Windows software.

Results

The phytochemical investigations of the leaves and stem extracts of the *P. minima* plant revealed the presence of alkaloids, flavonoids, tannin, phenols, terpenoids, steroids, proteins and cardiac glycosides. The result also recorded the absence of saponin, quinone and reducing sugar. That is shown in Table 1. To evaluate the anthelmintic activity ethanolic extracts of *P. minima* were used and which was found to dose-dependent. The data are expressed in mean ± SEM (statistical analysis) values and compared with control. The experimental results of anthelmintic activity in flatworm are given in Figs. 1, 2 which reveal that the leaves and stem extracts have shown paralysis and death of flatworms in different concentration, and it was compared with the reference drug Albendazole. Less time was required to cause paralysis (10.5 min for leaves and 11.3 min for stem) and a little more time to cause death (28.8 min for leaves and 20 min for stem) of worms by an ethanolic extract (100 mg/ml) as compared with reference drug (50 mg/ml).

Table 1 Phytochemical analysis in the ethanolic extract of *P. minima*

Name of the group	Leaves	Stem
Alkaloids	+	+
Flavonoids	+	+
Saponins	–	–
Tannins	+	+
Phenols	+	+
Cardiac glycosides	+	+
Terpenoids	+	+
Gums	–	+
Steroids	+	+
Proteins	+	+
Reducing Sugars	–	–
Quinone	–	–

(+) = Presence, (–) = Absence

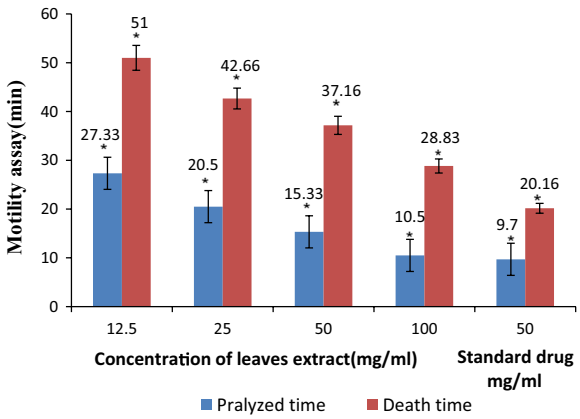

Fig. 1 Graphical presentation for anthelmintic activity of *P. minima* leaves on the basis of paralysis time and death time on *P. cervi* in different concentration and it was compared with standard drug Albendazole. Less time was required for leaves to cause paralysis and death (10.5 min and 28.83 min) of worms by an ethanolic extract (100 mg/ml). Data are expressed in mean ± SEM (*$p < 0.05$)

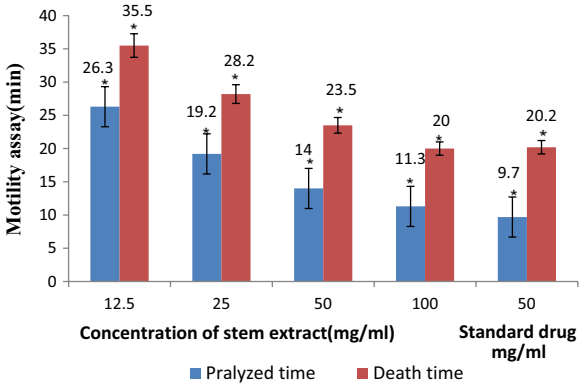

Fig. 2 Graphical presentation for anthelmintic activity of *P. minima* stem on the basis of paralysis time and death time on *P. cervi* in different concentration and it was compared with standard drug Albendazole. Less time was required for stem to cause paralysis and death (11.3 min and 20 min) of worms by an ethanolic extract (100 mg/ml). Data are expressed in mean ± SEM (*$p < 0.05$)

Discussion

Flaccid paralysis is exhibited by the effects of Albendazole on the worm that causes the result in a discharge of the worm. Albendazole kills the worms with rising chloride ion conductance in the muscle membrane of parasites which produces hyperpolarization and reduces excitability that resulting in muscle relaxation and nervous disorder. Paralysis as well as the death of the worms was confirmed by the plant extract at maximum concentration of 100 mg/ml when compared with Albendazole. Generally polyphenolic compounds show the anthelmintic activity and chemically tannins are one kind of polyphenolic compound. It may be possible that an active compound like tannins is found in the extract of *P. minima* which produces similar effects. Tannins show a significant anthelmintic effect because they have the ability to bind with free protein and glycol protein in the gastrointestinal tract of the host animal and parasite respectively (Kyriazakis et al. 2001). This behavior was similar to *A. muricata* aqueous leaf extract which showed the phytochemical analysis and anthelmintic effects (Ferreira et al. 2013). Several kinds of plant extract have been reported as anthelmintic activity (Tulasi et al. 2020). On the other hand, significant in vitro anthelmintic activity was exhibited by the methanolic extracts of leaves of *Sophora interrupta*. Albendazole was also used as a standard drug to compare the test results (Hemamalini et al. 2013). This behavior was observed in other plant extracts also. As, for example, an extract from *Corallocarpus epigaeus* tuber (Ishnava and Konar 2020) where Albendazole was used standard drug to compare the results. Our future plan of research work is the extract can be applied to a variety of other helmets which will be helped to find out the anthelmintic activity on a broader scale. The present study was carried out to evaluate the anthelmintic effect of *P. minima* on adult parasitic flatworm *P. cervi* (Trematoda) in cattle. In the present study, the ethanolic leaf and steam extracts of *P. minima* performed the major anthelmintic activity which was found to be dose-dependent inhibition of flatworm. This result was very significant when compared with the anthelmintic activity *of Sophora interrupta* methanolic leaf extract. It also exhibits the dose-dependent inhibition of spontaneous motility of earthworms (Hemamalini et al. 2013). The information obtained from the present

investigation is insufficient to use *P. minima* extracts as an anthelmintic agent, but the pure compound from *P. minima* extract might be a good subject in anthelmintic research due to the potent anthelmintic activity against the gastrointestinal parasite.

Conclusions

From the above result, it is concluded that the leaves and stem extracts from *P. minima* have promising anthelmintic effects when compared with conventionally used drugs. It is comparable with standard drugs. The preliminary phytochemical qualitative analysis of the ethanolic extract showed the presence of alkaloids, flavonoids, tannin, phenols, terpenoids, steroids, proteins and cardiac glycosides. Also recorded was the absence of saponin, quinone and reducing sugar. These extracts may be useful for the treatment of the helminthiasis. Further studies are required to find out and to establish the effectiveness and pharmacological motivation by using in vivo model for the use of the leaves and stem as an anthelmintic drug. Further studies are also required to isolate active constituents from the extracts and establish the mechanism of action.

Abbreviations

SEM: Standard error of the mean; PBS: Phosphate-buffered saline; HCl: Hydrochloric acid; NaOH: Sodium hydroxide; H_2SO_4: Sulfuric acid.

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Authors' contributions

FRSA and AS prepared the sample and analyzed the experimental assays. Anthelmintic activity measured by AS and FRSA. MJS and FRSA were responsible for writing reviews and edits. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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