

Effects of Ethanol Extract and its Different Fractions of *Phrynium imbricatum*(Roxb.) Leaves on In Vitro Anthelmintic and their Condensed Tannin Content

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Plant materials have been utilized for the treatment of serious diseases all through the world before the approach of advanced clinical medications. The utilization of therapeutic plants still assumes an essential part to cover the fundamental wellbeing needs in the developed countries. Most of the Phytochemical, secondary metabolites of plants, are physiologically active and these metabolites are known as to give an achieve wellspring of natural, anthelmintic, antibacterial and insecticides. Helminthic infestations are now being recognized as a cause of chronic ill health and sluggishness amongst the children. World Health Organization estimated 2 billion people infected with helminthes and it was also estimated that 100% of all age group of school children are at risk of morbidity. The major phyla of helminthes are nematodes (round worms) which are soil transmitted helminthes that mostly cause the intestinal infection, filarial worms cause the onchocerciasis and lymphatic filariasis, while platihelminths (flatworms) also known as trematodes like schistosomes and cestodes causes cyticercosis. Current estimates suggest that over half of the world population is infected with intestinal helminths, such as *Ascaris*, hookworms, *Trichuris*, *Enterobius*, *Strongyloides*, and tapeworms, and that most of these infected people live in remote rural areas in the developing countries. In case of other animals also gastrointestinal parasites causes infections that diminish the animal survival, growth rates and reproductive performance. Morbidity from nematodes is common with diabetes and lung cancer. The helminths parasites mainly subsist in human body in intestinal tract, but they are also found in tissue, as their larvae migrate towards them. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. Side effects of anthelmintic commonly include intestinal gastrointestinal disturbances nausea and giddiness, while various studies and reviews have showed the resistance to anthelmintic is increasing day to day. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity. In this regard, *Phrynium imbricatum* (Family: Marantaceae) is a rigid herb, which is commonly known as Pitulpata (Bangladesh). Leaves large, oblong, Spikes oblong, bracts oblong with obtuse, minutely toothed tips. Fruits usually 3-seeded. A paste prepared from leaves of *Phrynium imbricatum*, *Blumea clarkei* and an unidentified species (locally called Khedom gas) is applied to affected areas and bandaged for the treatment of fractures (Chakma). Leaves of *P. imbricatum* has activities like antiarthritic and membrane stabilizing. Occurs in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet. Leaves of *Phrynium imbricatum* (Accession No. 1315 CTGUH) were collected from Alu tila, khagrachari, Chittagong, Bangladesh in the month of September 2014 at the last time of its flowering. It is authenticated by Dr. Shaikh BU,

Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh. Leaves were cleaned with fresh water and dried for a period of 10 days under shade and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered of whole plant (850 g) of *Phrynium imbricatum* was soaked in 1.5 L ethanol for 7 days with occasional shaking and stirring and filtered through a cotton plug followed by Whatman filter paper number-1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (55 g) of the concentrated ethanol extract (EEPI) was fractioned by the modified Kupchan partitioning method into chloroform, CHFPI (8 g), n-hexane, NHFPI (6 g), ethyl acetate, EAFPI (9 g) and pat ether, PEFPI (14 g). The data on in vitro studies were reported as mean \pm S.E.M (n=3). Data were analyzed using one way factorial ANOVA tests using SPSS followed by Dennett's tests on each group except control for anthelmintic. Regression analysis was performed to calculate total tannin content. The anthelmintic activity of ethanol extract and its fractions of leaves of *Phrynium imbricatum* were carried out as per the procedure of Ajaiyeoba et al. with some minor modifications. The aquarium worm *Tubifex tubifex* were used in the present study because it has anatomical similarity and belongs to the same group of intestinal worm i.e., annelida. The worms were collected from the local market of Chittagong, average size of worms 2-2.5 cm. in length were used for the study. The standard drug Levamisole and three different concentrations of EEPI and its different fractions (5, 10 and 20 mg/ml) in double distilled water were prepared freshly and used for the study of anthelmintic activity. One group was composed of water and it was considered as controlled group. The anthelmintic activity was determine at two different stage 'time of paralysis' and 'time of death' of the worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors. Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased. Results of study were recorded as in the form of time required getting consecutive attacks of paralysis and at the end time required for complete death of parasite. From the observations made, higher concentration of extract and fractions produced paralytic effect much earlier and the time to death was shorter for all worms. From the above study it was seen that the ethanol extract showed dose dependent anthelmintic activity as compared to a standard drug Levamisole. Different treatment showed different anthelmintic activity. But ethanol extract of *P. imbricatum* showed highest anthelmintic activity. Where it paralyzed (3.69 ± 0.25 min); PCHFPI>EAFPI>NHFPI>PEFPI Ethanol extract of *Phrynium imbricatum* (EEPI) leaves showed

highest anthelmintic activity . The total phenol contents of the extracts . The total condensed tannin content of *Phrynium imbricatum* leaves was higher in plants at ethanol extract, which was 168.44 ± 0.87 mg catechin/g. Fractions of ethanol extract of *Phrynium imbricatum* contain good amounts of condensed tannin, ranging from 152.42 to 114.85 mg catechin/g. So condensed tannin content of ethanol and its fractions of *P. imbricatum* leaves are as follows, EEPI>CHFPI>EAFPI>NHFPI>PEFPI. Henceforth it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the proposal of screening medicinal plants for their anthelmintic activity. In this regard, *Phrynium imbricatum* (Family: Marantaceae) is a rigid herb, which is commonly known as Pitulpata (Bangladesh) . Leaves large, oblong, Spikes oblong, bracts oblong with obtuse, minutely toothed tips. Fruits usually 3-seeded. A paste prepared from leaves of *Phrynium imbricatum*, *Blumea clarkei* and an unidentified species (locally called Khedom gas) is applied to affected areas and bandaged for the treatment of fractures (Chakma). Leaves of *P. imbricatum* has activities like antiarthritic and membrane stabilizing . Occurs in the forests of Chittagong, Chittagong Hill Tracts, Cox's Bazar and Sylhet. Leaves of *Phrynium imbricatum* (Accession No. 1315 CTGUH) were collected from Alu tila, khagrachari, Chittagong, Bangladesh in the month of September 2014 at the last time of its flowering. It is authenticated by Dr. Shaikh BU, Associate Professor, Department of Botany, University of Chittagong, Chittagong, Bangladesh. Leaves were cleaned with fresh water and dried for a period of 10 days under shade and then powdered with a mechanical grinder, passing through sieve #40, and stored in a tight container. The powdered of whole plant (850 g) of *Phrynium imbricatum* was soaked in 1.5 L ethanol for 7 days with occasional shaking and stirring and filtered through a cotton plug followed by Whatman filter paper number-1. The extract was then concentrated by using a rotary evaporator at reduced temperature and pressure. A portion (55 g) of the concentrated ethanol extract (EEPI) was fractionated by the modified Kupchan partitioning method into chloroform, CHFPI (8 g), n-hexane, NHFPI (6 g), ethyl acetate, EAFPI (9 g) and pat ether, PEFPI (14 g). The data on in vitro studies were reported as mean \pm S.E.M (n=3). Data were analyzed using one way factorial ANOVA tests using SPSS followed by Dennett's tests on each group except control for anthelmintic. Regression analysis was performed to calculate total tannin content. The anthelmintic activity of ethanol extract and its fractions of leaves of *Phrynium imbricatum* were carried out as per the procedure of Ajaiyeoba et al. with some minor modifications. The aquarium worm *Tubifex tubifex* were used in the present study because it has anatomical similarity and belongs to the same group of intestinal worm i.e., annelida . The worms were collected from the local market of Chittagong, average size of worms 2-2.5 cm. in length were used for the study. The standard drug Levamisole and three different concentrations of EEPI and its different fractions (5, 10

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