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(Research Article)

In-Vitro Study of Cytotoxic, Anthelmintic and Antioxidant Activities of *Nymphoides hydrophylla*

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ABSTRACT

This study describes in vitro cytotoxic, anthelmintic and antioxidant activities of the leaves of Nymphoides hydrophylla belonging to the family Menyanthaceae. Powdered leaves of Nymphoides hydrophylla were treated with methanol for cold extraction. Crude methanol extract was used for brine shrimp lethality bioassay. Vincristine sulphate was used as standard. The crude extract showed positive result with LC_{50} value 3.2808 µg/ml for leaves compared to standard Vincristine sulphate with LC_{50} value 0.839µg/ml. Anthelmintic study was performed against Pheritima posthuma. Various concentrations of extracts were tested and results were expressed in terms of time for paralysis and time for death of worms. piperazine citrate was included as reference standard. In this study, methanol extract of leaves of Nymphoides hydrophylla exhibited significant anthelmintic property at higher dose (80mg/ml) compared to piperazine citrate at dose of 15mg/ml. The methanolic extract of Nymphoides hydrophylla was screened to calculate the total phenolic content for antioxidant activity by using Folin-Ciocaltu reagent, whereas Gallic acid was used as standard. Crude methanol extract has total phenolic content of 12.5±0.167 mg of GAE/gm. Thus, this study showed that crude methanol extract of Nymphoides hydrophylla leaves has significant cytotoxic activity, mild anthelmintic activity and moderate antioxidant activity.

Key words: *Nymphoides hydrophylla*, Cytotoxic activity, Anthelmintic activity, Antioxidant activity.

INTRODUCTION

Nature always acts as a great source of salvation for human being by providing different remedies from its plants, animals, and other sources to cure all ailments of mankind.Differenttypes of plant and plant derived compounds are used in folk medicine for the treatment of different ailments[1]. *Nymphoides hydrophylla is* an aquatic herb with floating leaves and long stem bearing tuft of roots at the nodes. Leaves 5-10 cm broad, orbicular, deeply cordate, purplish with green veins. Flowers densely fascicled at the nodes, corolla white, yellow towards the base within, 2 cm across when expanded, lobes 5-6, entire with a longitudinal fold down the middle. Capsule small, subglobose. This plant is native in Bangladesh, Bhutan, Cambodia and India, grows in in lakes, ponds and ditches. It is a annual sometimes perennial. Deeply rooted in soil Shallow freshwater ponds and slowly flowing water[2]. The plant is commonly used as a substitute for chiretta in the treatment of fever and jaundice. Stalks and leaves are pounded with oil and applied to ulcers and insect bites;decoction is used as a wash for parasitic skin affection. Seeds are considered anthelmintic. Leaves contain proteins and eleven amino acids including six essential amino acids[3].

MATERIALS AND METHODS

Collection of Plant

The plant *Nymphoides hydrophylla* was collected from surrounding area of Noakhali Science and Technology University, Sonapur, Noakhali in June, 2012.

Preparation of Extract

Leaves were separated from other parts and they were washed with water and air-dried under shade. After complete drying leaves were ground into fine powders. Fine powders were stored in a conditioned room until commencement of the test. Then about 330gm powered leaves of the experimental plant was taken in a container and soaked with 2100ml of 90% methanol. The whole mixture was occasionally stirred for about 14 days and then filtered through filter cloth. Obtained solvent was then evaporated at room temperature. Residue left at the bottom of the beaker after evaporation was crude methanol extract of leaves of *Nymphoides hydrophylla.*

Cytotoxic Activity Screening

In our present study, we used simple brine shrimp bioassay test which was developed by Meyer *et al*[4] using *Artemia salina* as test organism. First brine shrimp eggs are hatched in simulated sea water. Sample solutions are prepared by

dissolving test materials in precalculated amount of DMSO. 10 nauplii are taken in vial containing 5ml sea water. Samples of different concentration are added to different vials containing nauplii using micropipette. Survived nauplii are calculated after 24 hours. The data obtained are used to calculate LC50.

Anthelmintic Activity Screening

The anthelmintic assay was carried out as per the method of Ajaiyeoba *et al*[5] with minor modifications. Adult earthworms were used to study the anthelmintic activity due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings[6,7,8]. Because of availability of earthworms, they are widely used as effective tools for anthelmintic study[9,10,11]. Earthworms were collected from moist soil in the campus of Noakhali Science and Technology University. Collected earthworms were 3 – 5 cm in length and 0.1–0.2 cm in width weighing 0.8–3.04 g. They were thoroughly washed with saline water. Methanol extract of *Nymphoides hydrophlla* was used as test samples. It was used to prepare different concentrations (20, 40, 60 and 80 mg/ml) separately. 150mg of piperazine citrate was measured by weighing machine and dissolved in 10ml water to make a concentration of 15mg/ml. A control group was established with distilled water to ensure that the test was a validate one. Earthworms were divided into six groups each containing three earthworms. Four groups were used for methanol extract, one group was applied to reference standard and another to control group. For each concentration triplets (three petri dishes) were used, each petri dish containing equal sized earthworm.

Antioxidant Activity Screening

Phenolic compounds act as antioxidants by preventing the oxidation of Low-Density lipoproteins (LDL), platelet aggregation and damage of red blood cells[12]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[13]. Further, phenolic compounds are effective hydrogen donors, which make them antioxidant[14]. In the alkaline condition phenols ionize completely. When this ionized phenolic solution is treated with Folin-Ciocalteu reagent the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution becomes blue. The intensity of the color change is measured in a spectrophotometer at 760 nm. The absorbance value will reflect the total phenolic content of the compound[15].

Complete Ionization

$$\text{Phenols} + Na_2CO_3 \longrightarrow \text{Ionized phenols}$$

$$\text{Ionized phenols} + \underset{\text{Reagent}}{\text{Folin-Ciocalteu}} \longrightarrow \underset{\text{(Blue)}}{\text{Folin-Ciocalteu reagent complex}}$$
$$\text{(Yellow)}$$

In this study gallic acid was used as standard. To 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration and the total phenols content of the sample was measured. The phenolic contents of the sample were expressed as mg of GAE (gallic acid equivalent) / gm of the extract.

RESULTS AND DISCUSSIONS

Cytotoxic Activity

% Mortality of brine shrimps against logC for both Methanolic extract and Vincristine sulfate is shown in figure-1. Table-1 shows the result of cytotoxic activity screening.

Table-1: Results of the test sample of *Nymphoides hydrophlla*

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
Vincristine Sulphate (positive Control)	0.839	y = 34.02x + 52.58	R^2 = 0.952
Crude methanol extract (Leaves)	3.2808	y = 18.12x + 63.39	R^2 = 0.807

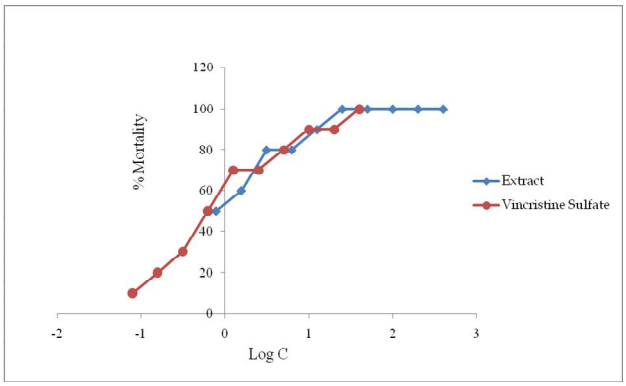

Fig. 1: Effect of Crude Methanol Extract and Vincristine sulfate.

The LC_{50} values of crude methanol extract of *Nymphoides hydrophylla* leaves found to be 3.2808 µg/ml. The positive control vincristine sulphate showed LC_{50} at a concentration of 0.839 µg/ml. From the results of the brine shrimp lethality bioassay it can be well predicted that the crude methanol extract of leaves of *Nymphoides hydrophylla* has cytotoxic property.

Anthelmintic Activity

Average time recorded for paralysis and death of earthworms for crude methanol extract of *Nymphoides hydrophylla* and standard drug are given in table-2 and graphically presented in figure-2.

Table-2: Paralysis and Death Time of *Pheretima posthuma* for Leaves Extract

Concentration (mg/ml)	Paralysis Time (Minutes)	Death Time (Minutes)
20 (Sample 01)	79 ± 1.154	83.33 ± 1.201
40 (Sample 02)	63.66 ± 0.666	68.00 ± 0.577
60 (Sample 03)	41.33 ± 0.881	45.33 ± 0.881
80 (Sample 04)	29.66 ± 0.881	35.33 ± 0.881
15 (Standard)	33.67 ± 1.53	51.33 ± 2.52
0 (Control)	0	0

Values are expressed as mean±SEM (Standard Error Mean), n=3

Crude methanol extracts of leaves of *Nymphoides hydrophylla* inhibited earthworms in a dose-dependent manner. The paralysis time of earthworms for leaves extract at different concentrations, including 20 mg/ml, 40 mg/ml, 60 mg/ml and 80 mg/ml was 79, 63.66, 41.33 and 29.66 minutes respectively, whereas death time was 83.33, 68, 45.33and35.33 minutes respectively. The paralysis and dead time for standard Albendazole at a concentration of 15 mg/ml were 33.67, and 51.33 minutes, respectively. This study showed that leaves of *Nymphoides hydrophylla* have anthelmintic property.

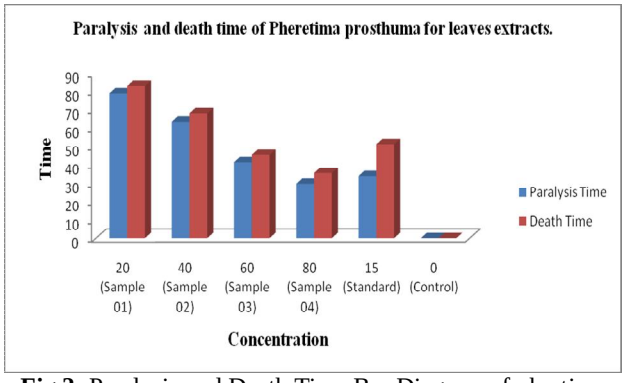

Fig.2: Paralysis and Death Time Bar Diagram of phertima prosthuma for Leaves Extract.

Antioxidant Activity

Based on the absorbance values of the various extract solutions the colorimetric analysis of the total phenolics of extracts were determined and compared with the standard solutions of gallic acid (y = 0.002x + 0.107, R^2 = 0.889) equivalents. Absorbance vs. concentration graph of Gallic acid is shown in figure 3. Total phenolic content of the samples are expressed as mg of GAE (gallic acid equivalent)/ gm of dry extract. The amount of total phenolic content in leaves of *Nymphoides hydrophylla* extracts are 12.5±0.67 mg of GAE / gm. Thus we can say the extract has moderate antioxidant activities.

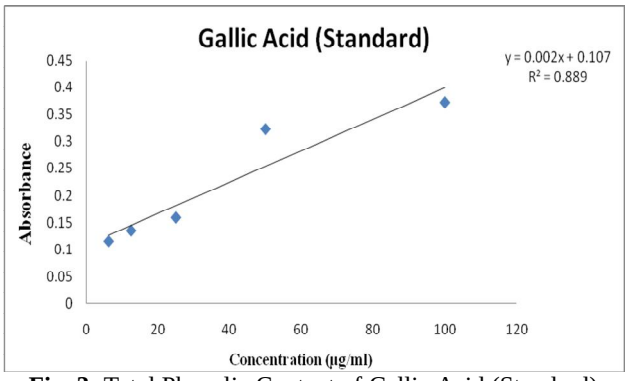

Fig. 3: Total Phenolic Content of Gallic Acid (Standard)

CONCLUSION

Investigation of *in vitro* cytotoxic activity of leaves of *Nymphoides hydrophylla* showed positive result. Crude methanol extract of test plant gave significant result compared to standard. The LC_{50} value of Crude methanol extract of Nymphoids hydrophylla is 3.2808(µg/ml) and for Vincristine sulfate LC_{50} value is 0.839. Control group showed 0% mortality which indicated validity of the study. These results concluded that *Nymphoids hydrophylla* leaves are cytotoxic.

Crude methanol extract of *Nymphoides hydrophlla* exhibited maximum efficacy at a concentration of 80 mg/ml. It proved mild anthelmintic activity of *Nymphoides hydrophlla.*

The methanolic extract of *Nymphoides hydrophylla* was subjected to screening for their possible antioxidant activity. The total phenolic amount was calculated (12.5 mg of GAE/gm of extract).In conclusion, it can be suggested that the crude methanolic extract of *Nymphoides hydrophylla* leaves possess moderate antioxidant activities.

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