A DETAILS STUDY ON HYGROPHILA DIFFORMIS

Samanta Krishanu*
Pharmacy College, Azamgarh, Uttar Pradesh, India.

ABSTRACT
The knowledge of medicinal plants must have been accumulated in the course of many centuries but it is our misfortune that proper chemical and pharmacological evaluation of most of these plants have not done till now. Keeping this view, a details study on Hygrophila difformis Blume (Family-Acanthaceae) along with phytochemical study have done. It is commonly known as water wisteria. It is a tropical aquarium plant used as environmental ornaments. It rapid growth helps prevention of algae. The plant grows to a height of 20-50 cm with a width of 15-25 cm, & slender lacy leaves and upright growth. It is found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan and Nepal. Hygrophiloside was found in the aerial parts of Hygrophila difformis. It is an iridoid glucoside having hepatoprotective activity. On preliminary phytochemical analysis Cardiac glycosides, tannins, steroids, flavonoids & saponins were found. It is used as coagulant by tribal people. The aerial parts of the plant showed good antioxidant property and also shows anthelmintic activity, Ethanolic extracts of aerial parts of the plant shows CNS depressant activity along with analgesic activity in mice.

Keywords: Hygrophila difformis, Antioxidant activity, Analgesic activity, Anthelmintic activity.

INTRODUCTION
Under the family Acanthaceae, Hygrophila difformis is commonly known as water wisteria. It is a tropical aquarium plant used as environmental ornaments. It rapid growth helps prevention of algae. The plant grows to a height of 20-50 cm with a width of 15-25 cm, and slender lacy leaves and upright growth. It is found in marshy habitats on the Indian subcontinent including Bangladesh, Bhutan and Nepal. It is easy to grow and as such it is a very popular plant for the tropical aquarium. It grows best in good light with nutriment rich water & it benefits from additional CO₂. It can be easily propagated from cutting. It is a beautiful plant for beginners which can help create a balance in the aquarium. Its rapids growth help prevent algae because the plants absorbs a great number of nutrients from the water. The storage of micro nutrients leads to pale leaves which may be indication that the aquariums need fertilizer. In large aquariums its lobed leaves can create a distinctive group. Out of 150 weeds species collected from rice fields of Hooghly and Midnapore districts of West Bengal, 124 species posses economic importance in one way or other. The various uses of those weeds may aid dealers in crude drugs, manufacture of plant products. The plant sometimes called water wisteria Hygrophila difformis is very fast growing plant that required high light and nutrients to get good green growth, when grown out of the water, the plant leaves change completely by becoming smaller and serrated with lots of
glandular hairs. When first submerged in an aquarium or pond this glands produce a toxic substances that may be toxic to some fish. So take great care when handling than as any damage seems to give off the toxin. Once they have been in the water a while the poison seems to disappear.

Flowers are about 1cm, light violet color. It was first described by Linnaeus himself (1781) as Ruellia difformis. It also called Ruellia triflora. Hygrophila difformis is a very important plant as a gift of the nature for living beings. It is used for administered by all type of herbivorous animals for their food. In traditional medicinal system it is used in different troubles as well as it possesses different important pharmacological activities. It is commonly known as Water wisteria or Wild wisteria.

It is widely distributed in India, Thailand, Malaysia, Burma & Bangladesh and Nepal. It is used as coagulant by tribal people it is an aquatic stem plant which grows mainly Asia minor & southeast Asia. It grows better in the rainy season than in the dry season. Leaves are about 1to 4 cm long. It can be identified by finely branched light green leaves above the water. A details theoretical survey regarding botanical description, traditional uses, chemical components, biological study was done in this paper.

**Taxonomical classification**
Kingdom – Plantae
Order – Lamiales
Family – Acanthaceae
Sub-family – Acanthoideae
Tribe – Ruellieae
Genus – Hygrophila
Specific eithet – Difformis B
Botanical name - *Hygrophila difformis*

**Vernacular names**
Dutch – Vaantjes plant
English – Water wisteria, Wild wisteria, Blue wisteria
Bengali – Bagua, kalaghass

**Habitat**
Maximum height – 20 – 50cm

Maximum length – 15 – 30cm
Temperature – 20 – 280C
Coloration – Bright green
Ph – 5.0 – 9.0
Water hardness – GH 7 – 13 dl.
Continent – Asia

**Chemical constituents**
*Hygrophila difformis* contain iridoid glucoside, hygrophiloside. Hygrophiloside apparently identical to the so called Cardanthera, Pseudoindican. Its structure has been established by spectroscopic means and by reduction to isoaucubin. S.R. Jensen and B.J. Nielsen, *Phytochem* (1985), 24 (3), 602

Preliminary qualitative phytochemical screening shown that presence of Cardiac glycosides, tannins, steroids, flavonoids and saponins.

**Member of the Genus Hygrophila**
Various genus of Hygrophila are given below
1. *H. guianensis*
2. *H. auriculata*
3. *H. dimidiate*
4. *H. philomoides*
5. *H. polysperma*
6. *H. quadrivalvis*
7. *H. salicifolia*
8. *H. schulii*
9. *H. angustifolia*
10. *H. balsalica*
11. *H. costata*
12. *H. corymbosa*
13. *H. lacustris*
14. *H. stricta*
15. *H. siamensis*
16. *H. aquatic*

**ANTIOXIDANT ACTIVITY**
The antioxidant activities of aerial part of *H. difformis* extract were investigated pal et al. by estimating degree of non-enzymatic haemoglobin glycosylation measured calorimetrically. Result obtained indicate the benzene & chloroform extract of aerial parts of *H. difformis* have better antioxidant activity then petroleum ether, ethanol & aqueous extract. The activities were comparing with D-α-tocopherol (Vit. E) & ascorbic acid (Vit.
C) that were used as a standard antioxidant compound. The detail chemical nature of the active principle responsible for the antioxidant activity & their mode of action are under investigation. Haemoglobin, 60mg/100ml in 0.01M, phosphate buffer (ph-7.4) was incubated in presence of 2gm/100ml concentration of glucose for 72 hours in required to best haemoglobin glycosylation. The assay was performed by adding 1ml glucose solution, 1 ml of haemoglobin solution & 1 ml of gentamycin (20mg/100ml) in 0.01M phosphate buffer (ph-7.4). The mixture was incubated in dark room temperature for 72 hours. The degree of glycolysation of haemoglobin in the present of different concentration of extracts and their absence were measured calorimetrically. The result indicate that the benzene and chloroform extract of aerial parts of \textit{H. difformis} have better antioxidant activity than petroleum ether, ethanol and aqueous extract.\(^4\)

Another investigates the antioxidant (in vitro) activity of methanolic extract of leaves of \textit{H. difformis}. Preliminary phytochemical test showed presence of phenolic compounds, flavonoids, tannins, steroids & also alkaloids. Antioxidant activity of methanolic extract was assessed by different in-vitro model of measuring anti-oxidant profile i.e. DPPH induced, superoxide & hydrogen peroxide induced method. Alterations of the absorbance due to free radicals were tested in normal, test (\textit{H. difformis} extract) & also standard(stock solution of vitamin C & sodium metabisulphite) groups.\(^5\)

Also investigate in vitro activity of leaf extracts of \textit{Hygrophila difformis} S L. Leaf part of the power was extracted with phosphate buffer and other leaf part of the extract was successively extracted with hydro-alcohol(water: ethanol::1:1)ethyl acetate & n-butanol measuring DPPH induced free radical scavenging activity, measurement of reductive ability, superoxide anion scavenging activity & hydroxyl radical scavenging activity.\(^6\)

**CNS ACTIVITIES**

CNS activities of aerial part of \textit{H. difformis} extract were investigated pal et al & Samanta et al. EEHD enhanced sleeping time, analgesic and anticonvulsant activities and reduced behavioral reflexes. Ethanol extract of aerial part of \textit{H. difformis} exhibited strong CNS depression action.\(^7\)

1. **Toxicity study**

Adult Albino Swiss mice of either sex weighing between 20-25gm were used. The animals were obtained from B.N. Ghosh & co, Kolkata. The animal were kept to normal laboratory condition for one week and given standard pellet diet (Hindustan Lever Ltd., Kolkata) and given water ad Libitum. The test compounds dissolved in water at different doses were injected intraperitronially to different group of mice. Each group containing ten animals.[Litchfield and wilcoxon]\(^8\)found that no mice died up to the dose of 2gm/kg. So EEHD may be considered as safe drug.

2. **Effect on sleeping time**

The mice were divided into four groups each group containing 6 mice. The animals of group-i received normal saline (0.9%w/v NaCl). Group-ii received EEHD at the dose of 400mg/kg, group-iii received EEHD at the dose of 500mg/kg and last Group-iv received EEHD at the dose of 600mg/kg respectively. Normal saline the extract were injected intraperitoneally 30 min prior to the administration of pentobarbitone sodium (40mg/kg, i.p) Diazepam (3mg/kg, i.p) & meprobamate(100mg/kg,i.p). The sleeping time was noted by recording the interval between the losses and regaining righting reflex.\(^9,10\)

3. **Analgesic properties**

A). **Acetic acid induce writhing method**

Acetic acid induce writhing method another name is chemical stimulus methods. The onset or writhing was noted. The number of abdominal constrictions, trunk twist response during a period of 10min was recorded. The mean writhing scores in
control, standard & test groups were calculated & % inhibition of pain in standard & test groups was calculated. This method used freshly prepared 1.2 % (v/v) acetic acid. The number of abdominal constrictions (writhing) stretching with a jerk at the hind limbs and bending of trunk were counted after administration of acetic acid. The results were compared with those of acetysalicylic acid, (68mg/kg), paracetamol (68mg/kg), and morphine sulfate (1.15mg/kg). The results revealed that the ethanolic extract of the aerial parts of *Hygrophila difformis* possess moderate central and peripheral analgesic activity.

**B). Hot plate method**

Thermal stimulus method evaluated by Eddy hot plate method. The mice are placed on the hot plate maintaining a constant temp. of 55°C the basal reaction time was noted against each mouse by observing hind paw licking or jump response whichever appears first. Normally such response time is 5-7 seconds. A cut off period of 30 sec. is observed to avoid damage to the paws. The reaction time was noted at 30, 45, 60, 90, 120, 150, 180 min. of drug administration. Morphine and pethidine were used as reference drugs (5, 10mg/kg, i.p, respectively) and also 15 min prior to the administration of reference drugs to investigate the potentiating of morphine and pethidine activity. Increase of reaction time after administration of Ethanol extract of *H. difformis*. So conclude that the extract may have some centrally acting analgesic activity.

**4. Anticonvulsant activity**

The anticonvulsant property on EEHD (200-500mg/kg, i.p) was tested strychnine (2mg/kg, i.p) and Leptazol (80mg/kg i.p.) induced conversant. The average survival time and percentage of mortality after 24 hrs recorded. Average No. of Jerking up to 15 min recorded. Jerking contain (Jump, major jump and minor jump). From the table it is found that EEHD the dose level 450mg/kg produced good anticonvulsant effect. The anticonvulsant activity EEHD may be due to enhance level of brain serotonin and GABA in mice.

**5. Behavioral effect**

The effects of EEHD (400,500 and 600mg/kg, i.p.) on rightning reflex, pinna reflex, corneal reflex, awareness, grip strength, touch, and pain responses on mice were observed conventional methods. Chlorpromazine (5mg/kg, i.p) was used as a reference drug. Number of animal used for each group is six. EEHD (600mg/kg i.p) showed very strong depression.

**ANTHELMINTIC ACTIVITY**

The Anthelmintic activity of aerial parts of *Hygrophila difformis* extracts was determined by samanta et al. worms were placed in Petri dishes containing 20 ml of different concentrations (10, 25 and 30 mg/ml) .The extract solution contains three each of petroleum ether (PEHD), benzene (BEHD), chloroform (CEHD) and ethanol extract (EEHD) respectively additionally with 1% gum acacia in normal saline at 37 ± 1°C. Albendazole (10mg/ml) was used as reference standard (positive control) while 1% gum acacia in normal saline as negative control. Six set of experiments was done for each concentration. The time taken to paralyze and kill individual worms was observed. Compare to all the extracts, the benzene extract (BEHD) was found to be most effective for paralyzing as well as for killing the worms and petroleum ether extract is having least anthelmintic activity. Maximum activity was found at dose of 30 mg/ml. Albendazole took moderate time for paralysis (37.17 ± 2.04 min) or death (65.50 ± 1.84 min) for the worms (p <0.001). The benzene extract of *H. difformis* displayed a significant anthelmintic property in a dose dependent manner giving shortest time of paralysis and death with 30 mg/ml concentrations, comparable with the standard drug albendazole. The methanol and chloroform extracts exhibited a modest activity.
HEPATOPROTECTIVE EFFECT
The methanolic extract of *Hygrophila difformis* (MEHD) Blume investigates hepatoprotective activity in rats by inducing liver damage by paracetamol. The methanolic extract at an i.p dose of 200mg/kg & 400mg/kg shows a significant protective effect by lowering serum levels of glutamic oxaloacetic transminase, glutamic pyruvic transminase, alkaline phosphatase and total bilirubin. LIV.52 used as positive control. The prevention of liver damage & curative effect of the drug was judged by changes in serum marker ALT, AST, ALP, protein & bilirubin levels. The plant phenolic compounds such as flavonoids have an important role in the treatment of many diseases & potent hepatoprotective effects.²⁵

TRADITIONAL USE
The leaves are used in traditional medicine for injury and cuts and for soothing purpose. It also used as coagulant by tribal people. Root posses expectorant property used in cough. The present article reviews a details study on *Hygrophila difformis*. The whole plant as well as it extracts both possesses the various pharmacological properties like Antioxidant activity, analgesic properties, anti-convulsant activity, Anthelmintic activity, Hepatoprotective effect.

CONCLUSION
A details study of the *Hygrophila difformis* Blume study concluded that it different extracts possesses the various pharmacological properties like antioxidant activity, analgesic properties, anti-convulsant activity, anthelmintic activity, Hepatoprotective effect. Phytochemical screening will also help the presence of compounds, which is responsible for the medicinal importance of the plant. *Hygrophila difformis* Blume. is known as wide range of medicinal value, it helps to identification, authentication and standardization. Although the different extract of the plant has numerous pharmacological importance but medicinal application and clinical application can be made only after extensive research on its bio-activity, mechanism of action, pharmacotherapeutics and extensive safety studies. It also require to research on pharmacognostical, phytochemical and pharmacological aspect. However research going on it would be easier to develop new drugs after extensive studies on mechanism of action & pharmacological effects. It is
expected that it may find application as a novel drug in the future to control various diseases.

ACKNOWLEDGEMENT
An author sincerely thanks to Dr. Dilip Kumar Pal, Associate Professor, Institute of Pharmaceutical Sciences, Guru Ghasidas Visvavidyalay (a central university). Koni, Bilaspur, Chhatrisgarh-495 009, India.

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