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Research Article

EFFECT OF WATER POLLUTION ON PHARMACOGNOSTIC PROPERTIES OF *HYDRILLA VERTICILLATA* (L.F.) ROYLE

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ABSTRACT

Hydrilla verticillata belongs to *Hydrocharitaceae* family is a noxious weed. The present investigation attempts to record the pharmacognostic properties of *Hydrilla verticillata* grown in polluted and unpolluted water sources. The morphological, microscopic and macroscopic studies serve as a standard reference for identification, authentication and distinguishing plant from its adulterants.
Keywords: Adulterants, *Hydrilla verticillata*, Identification, Pharmacognosy and Water pollution.

INTRODUCTION

Environment exerts tremendous influences on growth of morphological and structural changes underlying plant tissues. Adverse environmental conditions can cause plants structural damage and dysfunction in plants. The present study was carried to compare the impact of polluted condition as the pharmacognostic properties of *Hydrilla verticillata* with which grown in unpolluted conditions.

MATERIALS AND METHODS

The selected plant *H. Verticillata* collected from Asaripallam, Agastheeswaram Taluk, Kanyakumari District, Tamil Nadu, India (Elevation about 460 meters (Mean Sea Level) and are used to carry out the pharmacognostic properties.

Macroscopic Studies

Mature and healthy plants of *H. verticillata*are collected to study the morphological characters. By using hand lens in the field and dissection microscope in the laboratory, micro and macroscopic characters of the plants were recorded.

Microscopic (Anatomy) Studies

Collection and Preparation of Specimens

H. verticillata collected from unpolluted and polluted water sources of Asaripallam area Kanyakumari district, Tamil Nadu, S. India. Care was taken to select healthy plants of normal growth. The required samples of different plant parts were cut and fixed in a mixture of Formalin (5ml) + Acetic acid (5ml)+ 70% Ethyl Alcohol (90ml) solution. After 24hr. of fixing, the specimens were dehydrated with graded series of tertiary-butyl alcohol as per the method of Sass (1940)[1]. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60 ^{o}C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of Rotary Microtomes. The thickness of the sections was 10-12mm. De-waxing of the sections was done by customary procedure (Johansen, 1940)[2]. The sections were stained with toluidine blue as per the method of O'Brien *et al*. (1964)[3]. Wherever necessary, sections were also stained with safranin and Fast-green and IKI (Iodine Potassium Iodide) for starch.

Photomicrographs

Microscopic descriptions of plant tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Labphoto-2 microscopic unit. For normal observations, bright field microscope was used. For the study of crystals, starch grains and lignified cells, polarized light microscope was employed. Since these structures have birefringent property, under polarized light microscope they appear bright against dark background. Magnifications of the figures are indicated by the scale – bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esaa, 1964)[4].

RESULTS

Macroscopic studies

Characteristics of *H. verticillata* from unpolluted water source

Hydrilla verticillata, a submerged aquatic weed, collected from unpolluted water source was subjected to macroscopic analysis. *H. verticillata* roots are simple, long and slender;

usually buried in hydro-soil, but also form adventitiously at nodes; below the hydro-soil. The stems are horizontal, creeping, and stoloniferous. Leaves are verticillate, and along most of the stem, usually three to five in number per node. Apical portions of the stem usually have the nodes tightly clustered, with each vertical bearing up to eight leaves, and are strongly serrated with the teeth visible to the naked eye. Each leaf terminates in a small spine. The midvein is sometimes reddish in colour, and is usually armed with an irregular row of spines. The nodal scales (squamulae intravaginal) are small, paired structures at the base of the leaves and are lanceolate, hyaline, and densely fringed with orange-brown, finger-like structures called fimbriae. *Hydrilla* plants occur as two biotypes. They can be either dioecious, with flowers of only one sex being produced on a particular plant or monoecious, with flowers of both sexes present on the same plant. Flowers are imperfect (unisexual), solitary and enclosed in spathes. The female flower is translucent white, with three broadly ovate petals, of 1.2 to 3.0 mm long; the three petals alternate with the sepals that are much narrower and slightly shorter; Stigmas are three and minute; ovary is at the base of a long (1.5 to 10+ cm) hypanthium. Male flower is solitary in leaf axils. Mature flowers abscise and rise to the surface. Sepals and petals are similar in size and shape to those of female flowers. Each of three stamens bears a four-celled anther that produces copious, minute and spherical pollen. Fruits are cylindrical, about 5 to 10 mm long, usually with long, spine-like processes. Seeds are smooth, brown, usually five or less, 2 to 3 mm long and borne in a single linear sequence. Two types of hibernacula are produced a brown, bulb like type produced at the ends of the stolon and a green, conical found in axils of branches.

Characteristics of *H.verticillata* from polluted water source

Hydrilla verticillata grown in polluted water showed external morphological changes such as dark green coloured stem and leaf, reduction in stem width, reduced leaf size and increased internodal length as compared to plants grown in unpolluted water.

Microscopic (anatomical) studies

Microscopic characteristics of *H.verticillata* from unpolluted water source:

Stem: Detailed transverse section of *H.verticillata* stem collected from unpolluted water pond is shown in Plate-I; Photo - A. Transverse section of *H. verticillata*stem shows single layer of epidermis with radially elongated cells. The cells are thin walled without cuticle and stomata. The cortex is wide, consists of two layers outer cortex (hypodermis) are compact cells with small intercellular space. The rest of the cortex has aerenchyma cells with large air cavities which are separated from one another by uniseriate layers of cells. All the cells of the cortex have numerous chloroplasts. Endodermis and pericycle are not clear. The vascular system is very much reduced and mainly composed of phloem with only a few sieve tubes embedded in the parenchyma. The xylem is represented by the central cavity and resembles an air space. It is due to the reduction of several xylem elements. Mechanical tissues is absent in the stem.

Leaf: Detailed transverse section of *H. verticillata* leaf collected from unpolluted pond water is shown in Plate - I; Photo – C. The leaf section shows upper and lower epidermis. The upper epidermis has no cuticle and is made up of larger cells. There is no mesophyll. The lower epidermis has smaller cells and no stomata. The cells contain numerous chloroplasts. A reduced vascular strand occurs in the center.

Microscopic characteristics of *Hydrillaverticillata* from polluted water source

Stem: A transverse section taken from the middle part of the stem of *H. verticillata* from polluted water is showed in Plate–I; Photo- B. The transverse section of *H. verticillata* stem, from polluted water source, consists of single layer of epidermis, 8-9 layers of cortex and central vascular system surrounded by endodermis and pericycle. Epidermal cells are lightly compressed on one another. Cortex contains aerenchyma cells of different shapes and sizes (10-16 thin walled) with air spaces and many chloroplasts. Single layered endodermis and pericycle consists of isodiametric cells. Accumulation of certain pollutants in the form of black spots is noted in cortex and vascular bundle. Increase in size of aerenchymatous and shrinkage of cortical cells are also observed.

Leaf: The leaves of *H. verticillata* collected from polluted water are very small, thin and reduced (Plate-1; Photo – D). The upper epidermis does not have cuticle and stomata. The upper epidermis is made up of larger cells and the lower epidermis has smaller cells. The epidermal cells have little chloroplasts and there is no mesophyll. In the middle part, there is some areas are empty without any cells (vascular bundles are much reduced).

Different environmental conditions are always reflected on the plants. Environmental conditions in combination with resource availability appear as key factors in determining the distribution and functional characteristics of the species which inhabit a particular region. Industrial polluting agents, gas or solid are considered permanent aggression factors of air, soil and water quality. Due to the above factors, the life of plants subjected to a generalized stress, which most often, materializes through an ecological imbalance (Megdalena *et al.,* 2006)[5]. Plants usually adapt to high concentration of pollutants and unfavourable environmental conditions which is likely to result in different morphology and anatomy (Wyszkowski and Wyszkowska, 2003)[6]. In addition, due to anthropogenic activities, i.e. augmented pollution, soil deterioration etc. specific morpho-anatomical and physico-biochemical characteristics are results due to plants adaptation in stressful environments (Kovacic and Nikolic, 2005)[7].

Plants are living organisms which play a major role towards the environment and life itself. Plants and greenery depend on numerous roles to allow the growth of plants to successfully sprout. However, there are many factors affecting plant growth reducing their ability to grow at its greatest potential. Pollution is a problem that can spread far and wide from its original source. The polluted soil sources affect plants by damaging root cells, which leads to an inhibition of the transport of water and nutrients. The polluted soil also had a lower ratio of ions which was probably a reason for the lower rate of plant growth and lower rates of photosynthesis and root respiration.

Reduced plant growth in polluted soil could be linked to lower microbial activity as evidenced from reduction of the respiratory activity of soil microorganisms. Studies have shown that in degraded soils, microbial activity decreases and the composition of the soil micro flora changes[8,9]. Concentration of some secondary metabolites, such as phenolic compounds or enzymes, are often analyzed as an indicators of plant reactions to stress factors such as soil pollution with toxic ions[10-12].

Heavy metals in soil and water where plants are living are seen to demonstrate interactions between these heavy metals and the plants. On one hand, heavy metals show negative effect on plants by inhibiting the growth, damaging the structure, affecting physiological and biochemical activities and decreasing the functions of the plants. On the other hand, plants have their own mechanisms of resistance against the negative effects of heavy metals by combining heavy metals with proteins and developing enzymes and nucleic acids to detoxify heavy metal pollution.

Several reports suggests that the pollutants can cause a serious threat to the overall physiology of plants[13-15]. Plants were also seen to be polluted by heavy metals which are non-essential elements accumulated more amount, they will adversely affect the absorption and transport of essential elements and disturb the metabolism and have an impact on growth and reproduction (Xu and Shi, 2000)[16]. In early stages, heavy metals inhibit photosynthesis and growth, then inhibits the differentiation of reproductive organs and finally disturbs the nutrient transport and mobilization[17,18] noted a reduction in the root vitality of *Styloxanthesguianensis* in mine tailings, which prevents the absorption of inorganic nutrients and led to chlorosis, that significantly affected the growth.

Plants sensitive to pollutants can present changes in their morphology, anatomy, physiology and biochemistry[19-21]. The anatomical analysis of injuries caused by pollutants on plant species has been used in various studies to assess the real damage caused by pollutants[22-24]. Several authors have related the deleterious effects of pollutants on the anatomy and ultra-structural leaf characteristics[25,26]. The present study led to conclude that visual assessments alone were not sufficient to determine the real effect of pollutants. The occurrence of cellular injury in the absence of visual macroscopic symptoms in *H. verticillata* showed that anatomical investigations allowed a more precise injury diagnosis caused by pollutants as observed in Soyabean[22]. It would be important to emphasize that a more detailed physiological assessment should be made to evaluate the potential of these species as bio indicators of polluted environments since *H. verticillata* presented considerable structural and micro-morphological alterations in response to pollution.

The anatomical features vary greatly and are of significant value in many plants[27,28] reported that the anatomical characters are influenced by the environmental pollution. The modified structural attributes in different plant body parts are of supreme importance to cope with adverse circumstances (Cutler and Dave, 2007)[29]. As self-defense system developed in plants under contaminated condition, plants experience changes like increase in the number of stomata and trichrome per unit area which prove to be a support to the plant for their survival in the contaminated environment (Azmat *et al.,* 2009)[30]. In addition, increased epidermal thickness contributes significantly in tackling hazardous effect of heavy metal contamination (Gomes *et al.,* 2009)[31].

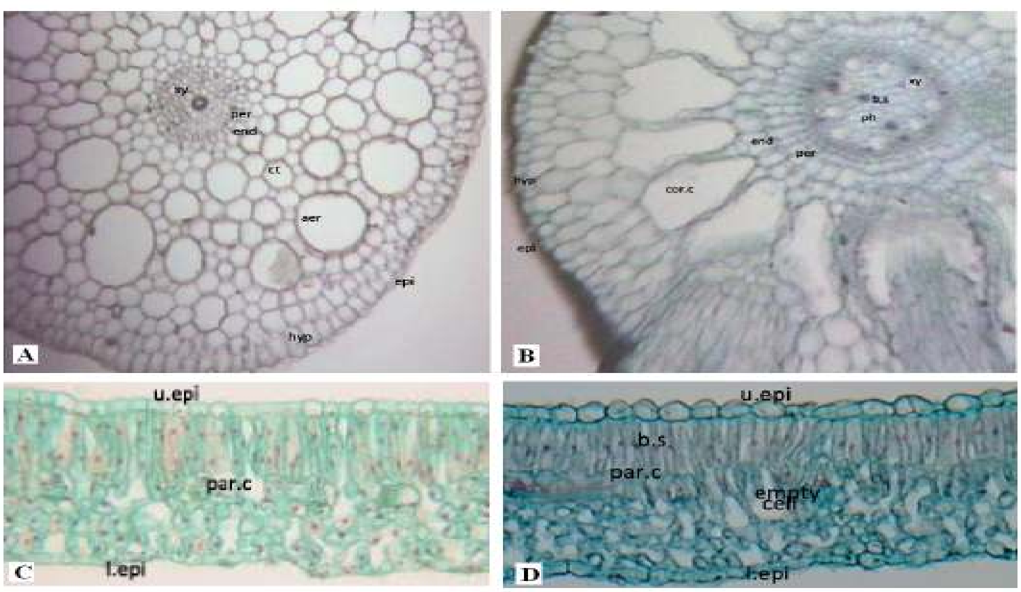

Plate I: Photo showing anatomical features of the stem (A & B) and leaf (C & D) of *Hydrillaverticillata* collected from unpolluted (A & C) and polluted (B & D) water sources.

epi	-Epidermis	Cor.c	- Cortical cell
hyp	-Hypodermis	b.s.	- black spot
aer	-Aerenchyma	u.epi	- upper epidermis
ct	-Cortex	l.epi	- lower epidermis
end	-Endodermis	par.c	- Parenchyma cells
per	-Pericycle		
xy	-Xylem		

CONCLUSION

Different environmental conditions are always reflected on the plants. Environmental conditions in combination with resource availability appear as key factors in determining the distribution and functional characteristics of the species which inhabit a particular region. Industrial polluting agents, gas or solid are considered permanent aggression factors of air, soil and water quality. Due to the above factors, the life of plants subjected to a generalized stress, which most often, materializes through an ecological imbalance. Plants usually adapt to high concentration of pollutants and unfavorable environmental conditions which is likely to result in different morphology and anatomy. In addition, due to anthropogenic activities,i.e. augmented pollution, soil deterioration etc. specific morpho-anatomical and physico-biochemical characteristics resultsdue to plants adaptation in stressful environments. It would be important to emphasize that a more detailed physiological assessment should be made to evaluate the potential of these species as bio indicators of polluted environments since*H. verticillata* presented considerable structural and micro-morphological alterations in response to pollution.

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