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Research Article

PHARMOCOGNOSTICAL STUDIES AND BIOPROSPECTING OF *BLEPHARIS SINDICA* STOCKS EX T. ANDERS

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ABSTRACT

Blepharis indica Stocks ex T. Anders is a herb of Acanthaceae family become "Vulnerable" due to climate change over use because of its medicinal properties. This plant is used for treatment of several ailments like kidney disorders, kidney stone, urine discharge, stomach pain, jaundice, diabetes, rheumatic pain etc. It is found only in the desert area like Rajasthan, Baluchistan (Pakistan), Saurashtra and Kutch (Gujarat) in India.

Key Words:

Blepharis indica, vulnerable,
bhangari, herb.

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INTRODUCTION

Blepharis indica Stocks ex T. Anders is a herb plant which is known with local name of Billy Khojio, Bhangari, Unt-kantalo and belong to the Family: Acanthaceae. *Blepharis indica* is about 45 cm height, dichotomously branched, woody annual plant. It is typical species with its spikes coming from near the base; make it quite a distinct plant. In dense associations the plant becomes erect and rather attractive. Its plant parts are valued for its medicinal, aromatic or savory qualities.

Medicinal plants, herbs, spices and herbal remedies are known to Ayurveda in India. A medicinal plant is any plant in which one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as medicinal plants (Suriyavathana and Kumar, 2010). There are more than 35,000 plants species being used in various human cultures around the world for medicinal purpose. Biologically active compounds present in medicinal plants have always been of great interest to scientist working in this field. It is a typical medicinal plant of Rajasthan that became vulnerable because of over use for its medicinal properties by local people. This plant is used for treatment of

several ailments like kidney disorders, kidney stone, urine discharge, stomach pain, jaundice, diabetes, rheumatic pain etc. Cream colored seeds are of medicinal importance. They are boiled in milk and used as an invigorating tonic. It is also given to cattle to increase milk production. Its roots are used for urinary discharge and dysmenorrheal. Powdered plant is applied locally on infections of the genitals and on burns. UNDP have published Red List Categories for 39 medicinal plants of Rajasthan State, out of which 19 are listed as Vulnerable, 12 as Endangered, 6 as Critically Endangered and one each of Near Threatened and Data Deficient, respectively. Out of these, *B. indica* is considered as "Vulnerable".

Because seed of *Blepharis* plant species of Acanthaceae family are dispersed by rain from the protected aerial seed banks adhere to the soil surface in rain flood water and only germinate after the excess water has reduced. All the seeds germinate after a short dispersal. Due to absence of soil seed bank for this plant, probability of availability of the new plants becomes rare. So this plant need broad range of research work for seed preserving methods and for multiplication of plant. Thus, it is very important to conserve this before it is eradicated. It is also necessary to study the adaptability of the plant to the present environmental conditions for its sustainment. Hence, in the present study, an attempt will be

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made towards the study of proline accumulation and its correlation with osmotic potential during different months of the collected plant material from various localities of the Churu district, a part of Indian Thar desert.

As this plant is an important with respect pharmaceutical point of view but being vulnerable it is high time that the plant should be protected through multiplication at large scale so that it is saved from being extinct and can be available for medicinal use.

Looking to the availability of information regarding this plant from internet and books, flora of state realized that basic studies of plant is also badly needed. As this plant is a wet herb little work has been done on this plant, because it is found only in the desert area like Rajasthan, Baluchistan (Pakistan), Saurashtra and Kutch (Gujarat) in India.

Few information about this plant are available that tell us about general anatomy, morphology and taxonomic characters includes that it is locally known as Bhangari and Unt-kantalo. It is a small, dichotomously branched plant, ash colored, teret finely pubescent plant. Leaves are sessile in a whorl of four, linear, acute usually with a few, spinous teeth near the base, margin recurved, midrib prominent beneath. Flowers in strobilate, subsessile, hairy spikes in the fork of branches. Calyx divided to base, softly hairy on both surfaces, outer segment largest, ovate. Corolla one lipped, the lip three lobed, mid lob quadrate, slightly larger than the lateral lobe which are rounded at apex. Stamen is didynamous, filaments are thick and rigid. Fruit is a capsule, ellipsoid, compressed, and narrowed at both ends and seed is also compressed (Bhandari, 1978).

Due to its limited localization the plant is unavailable to the mass for the studies on this plant. Hence present studies will be undertaken regarding identification and its biochemical characteristics. For rapid and mass multiplication of this plant tissue culture studies will be done. Plant tissue culture is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micro-propagation.

Plant tissue culture applied on the fact that many plant cells have the ability to regenerate a whole plant that are termed as totipotent cell and this ability is termed as totipotency. Single cells, plant cells without cell walls known as protoplast, explants of leaves, stems or roots can often be used to generate a new plant on culture media provided with the required nutrients and plant hormones. As already being a vulnerable species, attempts will be needed to genetically correlate the in vitro plant with in vivo plant to understand the genetic stability.

REVIEW OF LITERATURE

National Status

Blepharis sindica Stocks ex T. Anders is a typical medicinal plant of Rajasthan. This plant is used for treatment of several ailments like kidney disorders, kidney stone, urine discharge, stomach pain, jaundice, diabetes, rheumatic pain etc. Cream colored seeds are of medicinal importance. They are boiled in milk and used as an invigorating tonic. It is also given to cattle to increase milk production. Its roots are used for urinary

discharge and dysmenorrheal. Powdered plant is applied locally on infections of the genitals and on burns (Mohammad *et al.*, 2012)

Little investigation on the effect of ethanolic extract of *Blepharis edulis* Linn. (family - Acanthaceae) on general mating behavior, libido, and adverse effects on sexually normal male albino mice is also done. The suspension of the alcoholic extract was administered orally at the dose of 100, 250, and 500 mg / kg, to different groups of male mice (n = 6) once a day for seven days. The female Swiss Albino mice involved in mating were made receptive by hormonal treatment. The general mating behavior, libido and potency were determined and compared with the standard reference drug sildenafil citrate (Pande and Pathak, 2009). The results indicated that the ethanolic extract of *Blepharis edulis* Linn. produced a significant and sustained increase in hormonal levels of testosterone indication for the sexual activity of normal male mice without any adverse effects. Leaves of this plant reported to be useful in wounds, purgative disorder of liver and spleen, in asthma, throat inflammation, nasal hemorrhage and have contraceptive properties. In this plant constituent includes blepharin, benoxazolone, novel 4-o-glycoside of decarboxyrosmarinic acid (Pande and Pathak, 2009).

Inorganic ions such as Na⁺, K⁺ and Clamino acids, especially proline are major osmoregulator in some vacuole of osmolytes storage in large, osmotically inactive molecules such as starch or protein, which may serve several functions and from which they can be retrieved under conditions of stress investigated that Low temperature dose not result into the less absorption of water, however, it causes more rapid biosynthesis of proline, which results in lowering of osmotic potential (Ponvinobala *et al.*, 2012).

An attempt has been made to establish a correlation between proline accumulation and osmotic potential in *Blepharis sp.* Results revealed that proline accumulation is associated with the increase in osmotic potential in this plant. The maximum amount of proline was recorded during late season in November-December when the osmotic potential values were also more, whereas minimum proline was observed during onset of monsoon with initial phase of vegetative growth, i.e. in July- August (Mohammad *et al.*, 2012).

Phytosterols are cholesterol lowering agents represent a diverse group of natural products and knowledge about their occurrence in various plants and their chemical compositions have largely been studied. Variation in Phytosterols composition at various spatial and temporal events within semi arid part of the Indian Thar Desert were quantified. The study revealed that seed of *Blepharis sindica* stock ex T. Anders. possessed higher Phytosterols composition during winter season as compared to summer season. (Mathur, 2012).

The tissue obtained from a plant to be cultured is called an explant and may include portions of shoots, flowers, roots and single, undifferentiated cells. Based on certain model systems derived from work on tobacco and a few other plants, it has often been claimed that a totipotent explant can be grown from any part of the plant.

In India *in vitro* shoot development of *Blepharis repens* was achieved using thin sections of nodes and stems (1.0-1.5 mm) were cultured in MS medium containing varying

concentrations of different combination of phytohormones Indole-3-butyric acid (IBA), Benzylaminopurine(BAP) and Gibberlic acid. The combination of BAP, IBA and Gibberlic acid at different concentrations showed the length of shoot development 0.6 -1.8cm within three weeks in suitable laboratory (Suriyavathana and Kumar, 2010)

International Status

Plant secondary metabolites present chemical and pharmaceutical properties interesting for human health. Compounds belonging to the terpenoids, alkaloids and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases and in particular some of these compounds seems to be efficient in preventing and inhibiting various types of cancer (Rispaal *et al.*, 2003). Some work has been done in Pakistan on isolation and identification of plants of Acanthaceae (Ahmed Niyaz, 2012; Mohammad and Mohammad, 2011)

Phytochemical screening and biological activities of methanol extracts from three Acanthaceae species namely *Hygrophila auricula*, *Nelsonia canescens* and *Peristrophe bicalyculata* widely used in local ethno medicine, were carried out. Acetyl cholinesterase and Lipoxygenase inhibition tests revealed interesting activities from *P. bicalyculata*. Testing cancer cells antiproliferation, it was showed that *H. auriculata* and *N. canescens* were of good interest. Acetyl cholinesterase and Lipoxygenase inhibition tests revealed interesting activities from *P. bicalyculata*. Testing cancer cells antiproliferation, it was showed that *H. auriculata* and *N. canescens* were of good interest (Nabere, 2012). The aqueous-ethanolic extract of *Blepharis edulis* seed (Be.Cr) was studied for its antispasmodic and bronchodilator effect The Results were: Be.Cr tested positive for alkaloids, Flavonoids, tannins, sterols, terpenes, phenolic compounds and saponins (Fatima *et al.*, 2012).

It is reported that the antimicrobial activity of phenolic compound in *Blepharis edulis* is due to iron deprivation or hydrogen bonding with vital protein such as microbial enzymes. Research exhibited that *B. edulis* ethanolic extract (80% V/V) has shown no acceptable antimicrobial activity against bacteria and fungi and the MIC values of *B. edulis* against *S. aureus*, *B. subtilis*, *E. faecalis*, *P. aeruginosa*, *E. coli* and *A. niger*. In this study, 70% methanol extract has shown the best antimicrobial effect compared to ethanolic. Flavonoids possess antimicrobial activity against *C. albicans*, *Aspergillus flavus*, *A. tamari*, *A. flavus*, *Cladosporium shaerospermum*, *Penicillium digitatum*, *Penicillium italicum* extracts (Mohaddese *et al.*, 2001). Ethnoveterinary usages of Pakistan, desert plants has been studied by Khan (2009).

Light microscopic studies of *Blepharis attenuata* Napper showed that leaves have atriplicoid type kranz anatomy, where prominent BS and M form concentric tissue layers encircling leaf veins. In *Blepharis attenuata*, individual BS cells are enlarged, cuboidal, thick-walled, and compact containing numerous, large and centripetally-located chloroplasts. The surrounding Mesophyll tissue consists of thin-walled, radially arranged cells with fewer chloroplasts located around the periphery of the cell. In leaves of *B. attenuata*, the labeling for Rubisco, PEPC and PPdK is strictly compartmentalized between M and BS cells, typical of C₄ plants. Dense and specific labeling for Rubisco is present in the BS cells and

absent in the M cells (Muhaidat *et al.*, 2012). Single cell culture was first done by Haberlandt 1902. True callus with unlimited growth was first produced in 1939 by White. Earliest report of controlled shoot formation *in vitro* was provided by White (1939). Later white observations are confirmed by Skoog (1944), who showed that auxin could stimulate root formation and inhibit shoot formation. The discovery of kinetin (Muller *et al.*, 1956) led to new classical finding that both can regulate mechanism undergoing organogenesis evolved balance between auxin and cytokinin (Skoog and Miller *et al.*, 1957).

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