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Preliminary Phytochemical Analysis and Pharmacognostic Evaluation of *Atriplex stocksii* Boiss. (An Endemic Plant of Pakistan)

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Abstract: Plants are used in versatile by human beings. Metabolites produced by plants are used for medicinal purpose before any research on herbal medication, it is very important to analyze the standardization parameter of the plant. *Atriplex stocksii* Boiss. is an halophytic plant belongs to the family Chenopodiaceae. It is endemic in Pakistan. There is no report available on the pharmacognostic evaluation of *Atriplex stocksii* Boiss. therefore present research has been carried out. The present study provides an update information on its pharmacognostic, phytochemical and physico-chemical properties.

Key words: Phytochemical analysis · Pharmacognostic · Atriplexstocksii Boiss

INTRODUCTION

The importance of plants is well known to us. Plants gave comfort to mankind with respect to food, fuel, shelter and many other aspects. They produce not only food for surviving, but also create healthy environment and eco- friendly atmosphere. Use of plants for the medicinal purpose by humans is possibly as old as human civilization [1]. Plants produce many chemical compounds (phytochemicals) that have defensive properties for the protection against diseases [2]. Most important bioactive constituents of plants are steroids, terpinoids, flavanoids etc.

Nowadays, because of the development of modern and new sophisticated methods, scientists are taking more attention to explore new drugs from natural and biologically active compounds of the plants, which may serve as an endless resource for pharmaceutical industries. It is therefore necessary to establish a worldwide documented guiding principle for the evaluation and standardization of the quality of plants [3, 4]. The procedure of standardization of plants can be achieved by stepwise pharmacognostic studies. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical, physico-chemical and biochemical characteristics [5]. Accurate identification of the plant and quality assurance is an integral part to ensure reproducible quality of crude drug before including in the pharmacopoeia.

Atriplex stocksii Boiss. belongs to the family Chenopodiaceae is a short, erect, robust, much branched perennial shrub, 20-60 cm high is an endemic plant of Pakistan [6]. It is distributed in both inland and coastal marshes and deserts around Karachi, Pakistan [7].

There is no record ofpharmacognostical work on the leaves of *Atriplex stocksii* Boiss. The objective of the present study is to evaluate various pharmacognostic standards like microscopy, physico-chemical constant, fluorescence analysis and qualitative preliminary phytochemical analysis of *Atriplex stocksii* Boiss. these findings would be helpful for authentication, purification, quality control and for better use in pharmaceutical herbal formulations.

MATERIALS AND METHODS

Plant Selection: The leaves of *Atriplex stocksii* Boiss. was collected from the vicinity of the University of Karachi, Karachi. It was further identified and authenticated by the Centre for Plant conservation, University of Karachi. Voucher specimen was also deposited in (KUH) Herbarium, Centre for plant

Corresponding Author: Shazia Mansuri, Centre for Plant Conservation, University of Karachi, Karachi-75270, Pakistan. Tel: +92-21-99261369. conservation, University of Karachi for future reference no. 86547. The leaves were dried in the shade and was ground into fine powder.

Macroscopic Study: The fresh leaves of plants were taken for various macroscopic evaluations like color, odor, size and shape etc.

Microscopic Study: Qualitative microscopic evaluation was carried out by taking a transverse section of fresh leaves of *Atriplex stocksii* Boiss. fine section were selected mounted in glycerin and observed under compound microscope T.S of leaf, stomatalindex,type of Stomata, vein islet and vein termination number were carried out.

Physicochemical Analysis: A range of physicochemical parameters such as total ash, acid insoluble ash, moisture content were performed according to the standard methods given in the WHO guidelines for quality control methods for medicinal plant materials [8].

Florescence Analysis: The dried leaf powderwas observed under visible light and UV light of the range 366nm after treatment with different reagents like NaOH, HCL, H₂SO₄, Acetic acid, FeCl₃ and D.H₂o.

Phytochemical Analysis: Phytochemical analysis of the plant has been performed by using methods described by Raaman [9]. Preliminary phytochemical screening was carried out with the help of standard procedure described by Mayer's test [10] for alkaloids, Fehling test for carbohydrates, lead acetate test for phenolic compounds, alkaline reagent test for flavanoids, borntrager's test for glycosides, mucilage and gums, Biuret test [11] for proteins, Keller Kiliani test for cardiac glycosides, spot test [12] for fixed oils, Mucilage and Gums [13].

Statical Analysis: Standard error of the data have been calculated by using spss 12.0 version for windows.

RESULTS AND DISCUSSION

Whole plant shown in Figure 1. Its morphological characteristics reveals that the leaves of the plant are simple, both upper and lower surfaces contained anomotetracytic stomata (Figure 2). It has Unicellelar, unbranched trichome shown in Figure 3. Leaf constants



Fig. 1: Atriplex stocksii Boiss. Plant

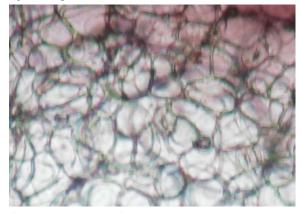


Fig. 2: Anomotetracytic Stomata

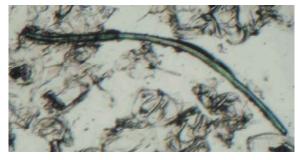


Fig. 3: Unicellular, Unbranched Trichome

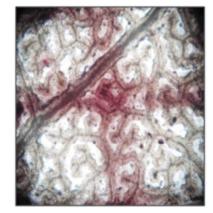


Fig. 4: Vein Islet and Termination

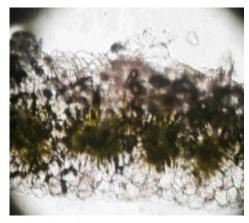


Fig. 5: T.S of Leaf

like, vein-islet number, vein termination number, stomatal number, number of epidermal cells, stomatal index were monitored and tabulated in the Table 1.

Various quantitative microscopy parameters such as stomatal number, vein-islet and vein termination number shown in Figure 4 was determined as per WHO guidelines. Results are given in Table 2.

Physico-chemical parameters are important for qualitative standards and useful in determining authenticity and purity of crude drugs. Total ash value, water soluble, acid insoluble is found to be 64.58 ± 0.25 , 47.9 ± 1.3 and 1.66 ± 0.33 respectively. Totalash value is relatively high which may be due to high contents of silicates, silica, contamination, substitution or adulteration [14]. Ash determination is helpful to judge the uniqueness and the cleanliness of the crude material. Water soluble ash is the measure of physiological inorganic components of the crude drug. Elevated water soluble content probably because of hard water supply which has excess high mineral contents [15]. Acid insoluble ash gives an idea about the non-physiological ash produced due to the adherence of inorganic dirt, dust to the crude drug. Increased acid insoluble means adulteration due to dirt, soil or sand [16].

Moisture is one of the important factors which are responsible for the deterioration of the drugs and formulations. Low moisture content is always needed for the higher stability of the drugs [17]. The moisture content is found to be 4.83%. The results of the physico- chemical parameters are given in Table 3.

Fluorescence study is an important parameter for the standardization of crude drugs. The characteristic fluorescent properties of the powdered leaves of

| BOISS. | |
|-----------------------|-----------------------|
| Parameters | Observations |
| Color | Greyish Green (shiny) |
| Odor | Odorless |
| Taste | A bit sour |
| Form | Simple |
| Shape | Ovate |
| Size | 1.5±0.05×0.96±0.03mm |
| Apex | Emarginate |
| Margin | Entire |
| Texture | Smooth |
| Venation | Reticulate |
| Base | Cuneate |
| Arrangement of leaves | Alternate |

Table 1: Morphological characteristics of the leaves of Atriplex stocksii

Table 2: Quantitative microscopic parameters of Atriplex stocksi iBoiss. leaf

| | Quantitative | | |
|------|------------------------|----------------------------------|--|
| S.No | microscopic parameters | Value | |
| 1. | Stomatal index: | | |
| | Upper surface | 19.74±0.99 | |
| | Lower surface | 20.5±1.84 | |
| 2. | Stomata type | Anomotetracytic | |
| 3. | Vein islet | 15 | |
| | Vein termination | 19 | |
| 4. | Trichome type | Unicellular, unbranched Trichome | |
| | | | |

Table 3: Physico- chemical properties of leaves of Atriplex stocksii Boiss.

| S.NO | Parameters | Values Obtained (% W/W) |
|------|---------------------------------|-------------------------|
| 1. | Moisture content | 4.83 ± 0.33 |
| 2. | Total ash value | 64.58 ± 0.25 |
| 3. | Water soluble | 47.9 ± 1.3 |
| 4. | Acid insoluble ash | 1.66 ± 0.33 |
| 5. | Extractive value in methnolic | 46 |
| 6. | Extractive value in choloroform | 4.7 |

Table 4: Fluorescence characters of leaves of Atriplex stocksii Boiss.,

| | Solvent | Under Ordinary | Under Uv Light |
|------|--------------------------------|-----------------------|-----------------------|
| S.No | Used | Light (After 1/2 Hr) | 366 Nm (After 1/2 Hr) |
| 1. | $Powder + D.H_2O$ | Light Green | Green |
| 2. | Powder + 1M HCl | Light Brown | Dark Brown |
| 3. | Powder + 1 M NaOH | Dark Yellow | Dark Yellow |
| 4. | Powder + H_2SO_4 | Green | Brown |
| 5. | Powder + 10% FeCl ₃ | Dark Green | Black |
| 6. | Powder + Glacial | | |
| | Acetic acid | Dark Brown | Dark Brown |

Table 5: Preliminary phytochemical analysis of different extracts of *Atriplex* stocksii Boiss.

| Phytoconstituents | Tests | Methanol | Chloroform |
|---------------------|-----------------------|----------|------------|
| Alkaloids | Mayer's test | + | - |
| Carbohydrates | Fehling test | - | - |
| Phenolic Compound | Lead acetate test | + | - |
| Flavonoids | Alkaline reagent test | + | - |
| Glycosides | Borntrager's test | - | - |
| Cardiac Glycosides | Keller Kiliani test | + | - |
| Fixed Oils and Fats | Spot Test | - | - |
| Proteins | Biuret test | - | - |
| Mucilage and Gums | Whistler and Be | | |
| | Miller, (1993) | - | - |

Atriplex stocksii Boiss. in daylight and UV light after treating with different reagents were recorded and presented in the Table 4. Many drugs fluorescence when their powder is exposed to ultraviolet radiation. It is important to observe all materials on reaction with different chemical reagents under UV light.

The results of preliminary phytochemical analysis of different extracts are given in Table 5. Secondary metabolites were found in good proportion in methanolic extract as compare to chloroform extract. Chloroform doesn't show the presence of secondary metabolites which means that the chloroform is not a good solvent for extraction of Atriplex stocksii Boiss.. Preliminary phytochemical revealed the presence of alkaloids, phenolic compounds, flavonoids, cardiac glycosides in the methanolic extract. Presence of phenolic compounds in the plants indicates that the plant may possess antimicrobial properties [18]. Flavonoids are effective water soluble antioxidants and free radical scavengers, which can prevent oxidative cell damage and exhibit anticancer effect [19]. Alkaloids play some metabolic role and control development in living systems. They are also involved in protective function in animals, have been used in insect studies and are used in medicine, contribute the majority of the poisons, neurotoxins and traditional psychedelic [20]. Cardiac glycosides are a class of natural products that are traditionally used to increase cardiac contractile force in patients. It is also useful in the treatment of asthma [21]. The role of cardiac glycosides in investigation on cancer is also reviewed [22]. Further investigation on the isolation and characterization of the antioxidant constituents is however required.

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