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**PHARMACOBOTANICAL, PHYSICOCHEMICAL AND PHYTOCHEMICAL
CHARACTERIZATION OF *ECHINOPS ECHINATUS ROXB.* (ASTERACEAE) LEAVES: A
COMPREHENSIVE PHARMACOGNOSTICAL STUDY**

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ABSTRACT

Systematic Study was carried out to determine pharmacognostic characteristics of *Echinops echinatus Roxb.* which is a plant species from Asteraceae family found in western ghats of Maharashtra, India. In this study all macroscopic, microscopic as well as physicochemical and phytochemical parameters of *Echinops echinatus Roxb.* are determined as per WHO Guidelines. Transverse sections of leaves have shown the presence of Upper and Lower Epidermis, Parenchyma, Collenchyma, Vascular bundle, Mesophyll, Trichomes and Stomata in it. Phytochemical investigation revealed the presence of flavonoids, alkaloids, glycosides in extracts of *Echinops echinatus Roxb.* Leaves. Physicochemical parameters such as ash value, extractive value, moisture content and fluorescent characteristics of leaf powder were also determined. In Present Study, we report the Pharmacognostic data and presence of flavonoids indicates high potential of this plant which is used as medicine for various diseases.

Keywords: *Echinops echinatus Roxb.*, Pharmacognostic Study, Flavonoids, Phytochemical Investigation, Asteraceae, WHO Guidelines

1. INTRODUCTION

Novel drug discoveries have shifted focus from synthetic models and compounds to natural products of plant origin. This is because scientists now believe that Lead molecules would be more

probably available in plants and other natural resources which are yet to be fully explored. Since long, a large number of plants are well documented in ancient medical literature of India.

Indian Traditional system of medicine is based on effective use of plants for the therapeutic and medicinal purposes. These plants typically from western ghats of India are widely known across the globe for their medicinal purposes [1]. *Echinops echinatus Roxb.* is one of the medicinal plant species which are found exclusively in western ghats of various states such as Maharashtra, Rajasthan India. It is an erect, short, spinous branched stems. Shrub was 50-90 cm in length. Branches were started from base of plant erect under shrub which is about 0.3-1 m tall annual plant. The Stems are woody, branches pubescent with stellate hairs. Leaves were alternately arranged, elongated. Greenish in colour. With length from 10-14 cm spines were present at corner of leaf. Leaf with marked midrib & rough surface. Spines were 2-7cm long. Leaf is so fibrous which on drying produces white thread like fibres or cotony structurts. to coarsel Apex was acute obtuse and laciniate hairy both above and beneath, petiole stellate hairy, Inflorescence of this plant is usually axillary, solitary, or in terminal subpanicles, by the reduction of the upper leaves. Flowers were like cotony balls, pedicel slender, stiff stellate hairy, jointed near apex, about 1.5-.2 cm long [2]. Leaves has been used by different cultures as a remedy for several diseases. It was also mentioned in Ayurveda as it was used in dysurea, for facilitating delivery, diabetes, thirst, cardiac disease, urolithiasis, fever, blisters of skin, sexual disability [3, 4]. We

hereby report about the pharmacognostic study of this species [5]. This study will be surely beneficial towards the standardization of the species and it will also be useful in determining the future scope in determination of its potential chemical constituents.

2. MATERIAL AND METHODS

2.1. Plant Material and Authentication

Plant of *E. echinatus* was gathered from hills of Manoli, Sangamner, Ahemadnagar Maharashtra, India. Authentication of collected plant material was carried out at Department of Botany From Sangamner Nagarpalika Arts, D. J. Malpani Commerce & B. N.Sarda Science College, Sangamner Ahemadnagar, Maharashtra, Voucher No.487 was deposited for further reference.

2.2. Morphological Evaluation

Morphological evaluation was carried as per WHO guidelines [5]. Important characteristics of plants such as Surface of Leaves, Colour, Odour, and Taste were evaluated.

2.3. Microscopic Evaluation

Transverse sections (T.S.) of *Echinops echinatus Roxb.* leaves were taken using sharp section blade. T.S. were kept in suitable media and then mounted on glass slide with the help of soft brush. T.S. were observed under Photographic Microscopes under Normal and Polarized Lights. Transverse Sections of leaves were studied for different microscopic characters like epidermis, xylem, phloem,

parenchymatous cells, Calcium Oxalate crystals, Starch grains etc. Photographs of all microscopic characters were captured, printed, labeled and stored for further referencing [6, 7].

2.4. Powder Microscopic Study

Preliminary examination and behavior of powder with different chemical reagents was carried out and microscopic examination was performed on treatment with different reagents like phloroglucinol, conc. HCl, ruthenium red, iodine solution and acetic acid. These are the reagents that are often used for detection of various microscopic components like xylem, phloem, calcium oxalate crystals, starch grains etc. Leaves of *Echinops echinatus Roxb.* were collected and shade dried. Dried leaves were powdered using a mixer grinder (preeti Ltd) and used further for microscopic evaluation. Powder was also treated separately with different chemical reagents and was observed under the microscope [7, 8].

2.5. Physicochemical analysis

Physicochemical parameters of *Echinops echinatus Roxb.* leaves powder such as ash values, extractive values, and moisture content (loss on drying) were determined according to methods prescribed in official books such as Indian Pharmacopoeia and the WHO guidelines on quality control methods for medicinal plant materials. Extractive values of *Echinops echinatus Roxb.* leaf powder were determined by using different solvents viz. water and ethanol. Five gram dried powder of leaves

were placed in a glass-stopper conical flasks containing different solvents. All the flasks were placed in a water bath shaker for 6 hours with frequent shaking, and allowed to stand for 18 hours. After 18 hours each extract containing different solvents were filtered and 25 ml of filtrate from each conical flask was dried at 105 °C for 6 hours and extractable matter of air-dried material was calculated.

The total Ash value was determined by burning ground leaf powder of *Echinops echinatus Roxb.* accurately 2.0 gm of powder was weighed in a previously ignited and tared silica crucible and it was ignited by gradually increasing the heat to 500-600 °C until it was red-white which indicate the absence of carbon. It was cooled in desiccators and weighed. The content of total ash in mg per gm of air-dried material was calculated. The Acid insoluble ash was determined by adding 25ml of hydrochloric acid (~70g/l) in to crucible containing 2.0gm of total ash. The crucible was covered and boiled for 5 min. The watch-glass was rinsed with 5.0 ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matter to the original crucible was dried on a hot-plate and ignited to constant weight. The residues were allowed to cool in suitable desiccators for 30 minutes and then weighed. The content of acid-

insoluble ash in mg per gm of air-dried material was calculated. The water soluble ash was determined by adding 25ml of water in to the crucible containing 2.0gm of total ash and it was boiled for 5 minutes. Insoluble matter was collected on an ash-less filter-paper, washed with hot water and ignited in a crucible for 15 minutes at a temperature not exceeding 450°C. The content of water-soluble ash in mg per gm of air-dried material was calculated by subtracting the weight of this residue in mg from the weight of total ash. The water soluble ash value was calculated. Loss on drying study was carried out by using hot air oven. The powder was taken in porcelain dish and weight of

empty porcelain dish was noted. After that 1 gm of powder was taken and allowed for 2 hours at 105°C for drying. The loss on drying was calculated [8].

2.6. Fluorescence analysis

Fluorescent characteristics of the plant powder as such and after treating them with chemical reagents were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the standard methods. Behavior of powdered plant materials with different chemical reagents was carried out as described by standard procedures [9].

| Treatment with chemical reagent | Observations | | |
|---------------------------------|---------------|-----------------------|---------------------|
| | Visible Light | Short uv 254 nm light | Long uv 365nm light |
| Water | Brown | Green | Green |
| Sodium hydroxide | Brown | Green | Green |
| Methanol | Green | Green | orange |
| Sulphuric acid | Pale yellow | Green | Green |
| Hydrochloric acid | Brown | Green | orange |
| Nitric acid | Brown | Green | Green |
| Ferric chloride | Brown | Green | Green |
| Acetic acid | yellow | Green | Brown |
| Picric acid | Green | Green | Yellow |
| Powder as such | Green | Green | Green |

2.7. Extraction process

The extraction of dried powder of the leaf of *Echinops echinatus Roxb.* was performed using maceration and soxhlet extraction process. 100 gm of coarsely powder air-dried leaf powder was successively extracted with different solvent 350 ml of petroleum ether (40-60°C) for 6 hr., 350 ml of chloroform (40-50°C) for 5-6 hr., 350 ml of methanol (60°C) for 18 hr., and 350 ml of water at

temperature (80°C) for 6 hr. in soxhlet extractor. Separate the extract and solvent was recovered in rotary evaporator. Cool and store the extract in desiccator and calculate the percentage yield of extract [11].

2.8. Preliminary phytochemical screening of extracts

Extracts of *Echinops echinatus Roxb.* were subjected to preliminary phytochemical screening for the

detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids. This examination was done by using standard

procedures [11].

3. RESULTS AND DISCUSSION

3.1. Morphological evaluation

Morphological characteristics of *Echinops echinatus Roxb.* are mentioned in Table 1.

Table 1: Morphological Characteristics of *Echinops echinatus Roxb.*

| Characteristics | Observation |
|-----------------|---|
| Surface of leaf | Wooly with spikes |
| Colour | Green |
| Odour | Pungent |
| Taste | Bitter |
| Size | 10-14 cm #1-2 cm |
| Shape | Pinnatifid amplexical with triangular projections forming spines. |

3.2. Microscopical characterization

Transverse section of Leaves of. was found to have anomocytic type of stomata i.e. Irregular celled stomata were present. The upper and lower epidermis consists of single row of cells with both glandular and multicellular trichomes. Spongy parenchymatous cells were also present in the intracellular spaces. Collenchyma along with clustered crystals of calcium oxalate was present in the section.

3.2.1. Powder microscopy

Powder analysis plays a significant role in identification of crude drug. These characters always help in the identification of right variety and search for adulterants. The microchemical test of leaf powder reveals the presence of Multicellular trichomes,

3.2.2. Physicochemical Evaluation

Results of various physicochemical parameters viz. Ash, extractive values and loss on drying are summarized in Table 2 [17, 18].

3.2.3 Preliminary phytochemical evaluation

Phytochemicals play an important role in the treatment of different types of diseases and disorders and are still used in both traditional and modern medicine. Many of the secondary metabolites isolated from plants are used in pharmaceutical industry. Ethanolic extract of *Echinops echinatus Roxb.* leaves showed presence of alkaloids, Carbohydrate, Tannins, flavonoids, glycosides and Phenolics. Results are discussed in the table along with its colour, odour, appearance and percentage yield (Table 3, 4).

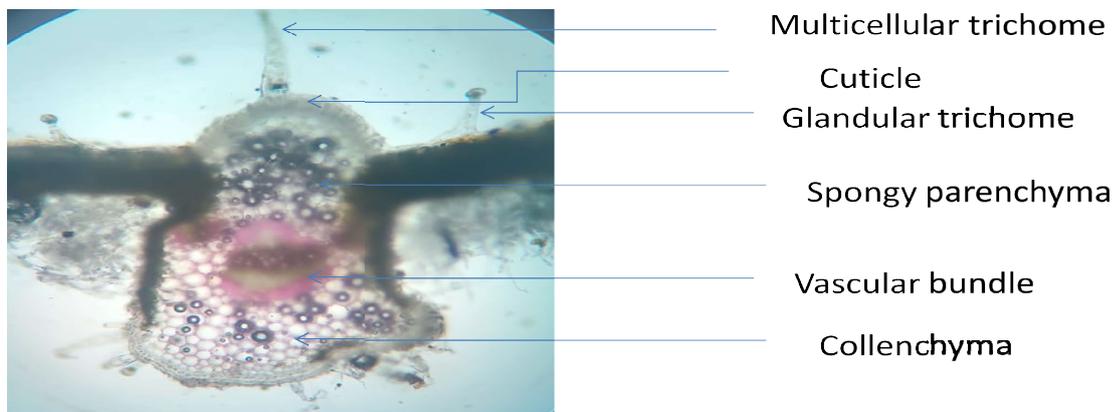


Figure 1: T.S. of Leaf of *Echinatus echinops* Roxb.



Figure 2: Anomocytic stomata

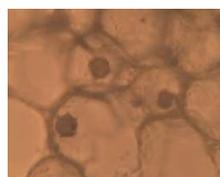


Figure 3: Calcium oxalate crystals



Figure 4: Xylem vessels, Fibres [15, 16]

Table 2: Physicochemical Evaluation

| Sr.No. | Parameters | Values in %w/w |
|--------|--------------------------------------|----------------|
| 1. | Moisture content determination (LOD) | 6% |
| 2. | Ash value | |
| | Total ash value | 9.5% |
| | Acid insoluble ash value | 1.5% |
| | Sulfated ash | 3.5% |
| | Water soluble ash value | 3% |
| 3. | Extractive values | |
| | Water soluble extractive | 6.4% |
| | Alcohol soluble extractive | 4.4% |
| 4. | Foreign organic matter | 1.98% |

Table 3: Extraction with various Solvents

| Sr.no. | Solvent used for extraction | colour | Odour | Apperance | % yield W/W |
|--------|-----------------------------|------------|----------------|-----------|-------------|
| 1. | Pet ether | Dark green | disaggregable | Sticky | 2.6% |
| | Benzene | Dark green | disaggregable | sticky | 2.54% |
| 2. | Chloroform | brown | characteristic | semisolid | 3.92% |
| | Ethyl acetate | Brown | pungent | semisolid | 4.69% |
| 3. | Methanol | Green | pungent | Semisolid | 6.3% |
| 4. | Water | Brown | pungent | Semisolid | 7.62% |

Table 4: Phytochemical Investigation of various extracts

| Nature | Pet ether extract | Chloroform extract | Methanol extract | Aqueous extract |
|-----------------------------------|-------------------|--------------------|------------------|-----------------|
| 1. Alkaloids | | | | |
| Mayer's test | - | + | + | + |
| Wagner's test | - | + | + | + |
| Hager's test | - | + | + | + |
| Dragendroff's test | - | + | + | + |
| 2. Carbohydrate | | | | |
| Molish's test | - | - | + | + |
| Fehling's test | - | - | + | + |
| Benedict's test | - | - | + | + |
| Barfoed's test | - | - | + | + |
| Bial's test | - | - | + | + |
| Aniline acetate test | - | - | + | + |
| Cobalt chloride test | - | - | + | + |
| Iodine test | - | - | + | + |
| Tannic acid test | - | - | + | + |
| 3. Flavonoids | | | | |
| Ferric chloride test | - | - | + | + |
| Shinoda test | - | - | + | + |
| Alkaline reagent test | - | - | + | + |
| Lead acetate solution test | - | - | + | + |
| Sodium hydroxide test | - | - | + | + |
| 4. Amino acids | | | | |
| Ninhydrin test | - | - | - | - |
| 5. Steroids | | | | |
| Salkowski test | + | + | + | + |
| Liebermann burchard test | + | + | + | + |
| 6. Tannins & phenolics | | | | |
| Ferric chloride Test | - | - | + | + |
| Gelatin test | - | - | + | + |
| Lead acetate test | - | - | + | + |
| Potassium dichromate test | - | - | + | + |
| Dil. Pot. Permanganate test | - | - | + | + |
| 7. Fats and oils | | | | |
| Spot test | + | - | - | - |
| 8. Volatile oil | | | | |
| | - | - | - | - |

4. CONCLUSION

Pharmacognostic and phytochemical investigation of *Echinops echinatus* Roxb. leaves was carried out.

The results obtained in this study will be useful

to authenticate the medicinal importance of the particular species of *Echinops*. Pharmacognostic parameters determined in present study will also be useful for establishing the pharmacopoeia

standards for *Echinops echinatus* Roxb. Preliminary phytochemical analysis will surely be useful for further phytochemical studies and isolation of therapeutically important phytoconstituents.

5. REFERENCES

- [1] Zunjar, V., et al. "Pharmacognostic, physicochemical and phytochemical studies on *Carica papaya* Linn. leaves." *Pharmacognosy Journal* 3. 20 (2011): 5-8.
- [2] Maurya, Santosh Kumar, Ashwini Kumar Kushwaha, and Ankit Seth. "Ethnomedicinal review of Usnakantaka (*Echinops echinatus* Roxb.)." *Pharmacognosy reviews*, 9. 18 (2015): 149.
- [3] Mujawar, F. A. A., et al. "Pharmacognostical, phytochemical and pharmacological of *Echinops echinatus* Roxb: a comprehensive review." *World J. Pharm. Sci.* 3 (2015): 1626-1632.
- [4] Kasote, Deepak M., et al. "Herbal remedies for urinary stones used in India and China: a review." *Journal of ethnopharmacology*. 203 (2017): 55-68.
- [5] World Health Organization. *Quality control methods for medicinal plant materials*. World Health Organization, 1998.
- [6] Khadabadi, S.S., S.L. Deore, and B.A. Baviskar. "Experimental phytopharmacognosy." *Nirali prakashan*, page 47 (2011).
- [7] Khandelwal, KR. *Practical pharmacognosy*. Pragati Books Pvt. Ltd., 2008.
- [8] Mukharjee, Pulok K. "Quality control of herbal drugs." *Business Horizons, Pharmaceutical Publishers* 40 (2002).
- [9] Tanwar, S, et al. "Standardization and phytochemical evaluation of *Tinospora cordifolia* (willd.) Miers. (Meni-spermaceae)." *Int J Pharm Pharm Sci* 4 (2012): 219-23.
- [10] Roy, Priyanka, et al. "Pharmacognosy and phytochemical screening of some plant derived medicine to treat dysmenorrheal pain by the Rajbanshi Community." *Pharmacognosy Journal* 10.4. (2018).
- [11] Nduche MV et al. "Phytochemical Screening & Antimicrobial activity of *Carica papaya* L. *Citrus paradisi* L. *Citrus sinensis* L, and *Vernonia amygdalina* Del. Cent", *Journal of Dairy & Veterinary Sciences*, 9(3), (2019).