

Pharmacognostical, Phytochemical and Pharmacological investigation of *Echinops echinatus* (Roxb.)

1. INTRODUCTION

1.1 Herbal drugs and evaluation of herbal drug

History of medicine goes back practically to the existence of human being. The current accepted Modern Medicine or allopathy has gradually developed over the years of scientific and observational efforts of scientists-however, the basis of its development remains in the roots of traditional medicine and therapies. Plants are the most exclusive source of drugs for the majority of World's population. Global estimate indicates that 80% of about four billion populations are using traditional medicines, which are mainly derived from medicinal plants listing over 20,000 species. Even in the allopathic medicine 25% of the prescription, substances are derived from higher plants. India is perhaps the largest producer of medicinal herbs and is rightly called the "Botanical Garden of the World". Except very few, all medicinal herbs of commercial importance are collected or cultivated in this country. Medicinal herbs have been of use for thousands of years in one form or another under the indigenous systems of medicine like Ayurveda, Siddha and Unani. Since independence in 1947, India has made tremendous progress in Agro technology, process-technology, standardization, quality control, and research and development etc. The WHO also appreciated the importance of medicinal plants for public health care in developing nations and evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy.

1.2 *Echinops echinatus* (Roxb.)

Family: Asteraceae

Part used: Whole plants, Roots, Seeds and Leaves

Vernacular names:

- English: Indian Globe Thistle
- Gujarati: Shuliyo, Utkanto, Utkato
- Hindi: Gokhru, Uthkanta, Utakatira
- Sanskrit: Kantalu, Kantaphala, Utati, Utkantaka, Utkatotkata, Vritttaguchha

Distribution: More or less throughout India and Afghanistan.

Description: *Echinops echinatus* is an erect branched herb about a meter high. It has short, stout stems, branching from the base, covered with white cottony hair.

Leaves: Alternately arranged oblong, deeply pinnatifid leaves are 7-12 cm long.

Flowers: Flower-heads occur in solitary white spherical balls, 3-5 cm across. Petals of the tiny white flowers are 5 mm long. Flowers are surrounded by straight, strong and white. Odour is pungent and taste is bitter.

Uses: The plant is pungent, bitter and hot, improves the taste and cures kapha and vata. It is used in urinary disorder, liver disorder, heart diseases, etc. The root is abortifacient aphrodisiac. The seeds are sweet and aphrodisiac (Aurveda).

The plant is bitter, stomachic, antipyretic, analgesic, increases the appetite stimulates the liver, useful in brain disease, used in ophthalmia, chronic fever, pains in the joints, inflammations, the root is aphrodisiac (yunani).

The drug is bitter and is consider being tonic and diuretic. The roots are pounded and mixed with acacia gum and applied to the hair to destroy lice. The powder roots are also applied to wounds in cattles to destroy maggots (Hughes-Buller).

1.3 Advantages of Herbal drug

- Their effectiveness, easy availability, low cost, and comparatively being devoid of serious toxic effects (time tested) popularized herbal remedies.

1.4 Rationale of Herbal Medicine

The traditional medicine is larger popularity over allopathic medicine because of the following reasons:

- Rising costs of medical care.
- As these are from natural origin, so free from side effects.
- Goes to root cause and removes it, so that the disease does not occur again.
- Cure from many obstinate disease.
- Easy availability of drugs from natural sources.

1.5 Role of a Pharmacognosist in promoting herbal drugs

- Although the roots of herbal medicine or drug have been traced back to thousands of years and most of its history, herbalist has been linked with religion, astrology, and superstition, but there is also a purely scientific approach to the world of herbs known as ‘Pharmacognosy’.
- A pharmacognosist has an expert knowledge of chemical constituents of plants, how to go about identifying new chemicals and even molecules that occur in plants, and how various cultures use plants for their benefits, with particular interest in their medicinal applications. Being a rationalist, only a pharmacognosist is interested in isolation, purification, characterization and describing the active ingredients, or bioactive molecules of plants. And only his or her investigations might lead to synthesis of bioactive molecules, or to achieve certain desired effects, such as increased activity, less toxicity or greater stability.

1.6 Steps necessary for isolating herbal drugs

Phytochemistry or natural product chemistry research is the backbone of herbal industry and directly or indirectly responsible for both failure and success of herbal drugs. For promoting the use of herbals in modern medicine, phytochemistry should be envisaged for:

- Isolation, purification and characterization of new phytoconstituents.
- Use of newly isolated phytoconstituent as “lead” compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity.
- Conservation of lead phytoconstituents into medicinally important drugs.

1.7 Problems and Challenges for Herbal Drugs

One of the major barriers to the successful outcome of drug discovery program from plants is the periodic need to obtain recollections of plant material, whether for structural confirmation of active constituents found in very low concentration in the plant part under study, or for the generation of larger quantities for biological or clinical testing. A typical example, wherein the generation of continued supplies of an active plant secondary metabolite is both crucial and uncertain is that of ‘taxol’. It has been estimated that 25,000 lbs (11,354 kg) of bark from 2500

trees are needed to supply one kg of taxol. Taxol has so far proven to be refractory to total synthesis, which is not surprising since it has 11 stereo centers, although the partial synthesis of this compound has been accomplished from several precursors. A potential approach towards solving the supply problem of taxol and other natural compounds obtained from threatened species are the selection and propagation of high yielding phenotypes and plant tissue cultures (PTC). In addition, it is also possible that less structurally complex taxol analog will be discovered having the same type and potency of antineoplastic activity as the parent compound and can then be synthesized.

The current alarming rate of species extinction is rapidly depleting the natural product treasury, with potentiality disastrous consequences. Hence, the need of the hour is to develop herbal gardens throughout the country, through individual and organizational efforts. On the other hand, the problem of obtaining a large-scale supply of promising plant-derived compounds may be overcome by plant tissue cultures.

2 LITERATURE REVIEW

Bhadoriya U *et al*¹ (2010) studied Diuretic activity of methanolic extract of leaves of *Salvadora persica* L. The diuretic effect of the extract was evaluated by measuring urine volume & sodium and potassium content. Urine volume was significantly increased by methanolic extract in comparison to the control group, while the excretion of sodium was also increased by extract. The methanolic extract had the additional advantage of a potassium-conserving effect.

Sravani P *et al*² (2010) evaluated Diuretic Activity of *Xanthium strumarium* L. The diuretic effect of the extract was evaluated by measuring urine volume, sodium and potassium content. Urine volume is significantly increased at two doses of PEXS 250 & 500 mg/kg body wt in treated rats. The excretion of sodium, Potassium levels was also increased by the PEXS.

Priyanka V and Rekha V³ (2010) studied Analgesic, anti-inflammatory and antipyretic activity of *Cissus quadrangularis*. The results showed that the ethanolic extract significantly reduce the edema induced by carrageenan within 1 to 5 hrs. post dosing at all the dose levels used. On the analgesic property acetic acid induce writhing was significantly reduce in the formalin test, the extract also significantly decreases the painful stimulus in both phases of test which confirms central and peripheral effects of the drugs.

Anuj KA *etal*⁴ (2010) Evaluated analgesic activity of methanolic extract of *Trapa natans* l.var. *Bispinosa* roxb. Roots. In tail flick method, the extract at 200mg/kg showed significant activity (P<0.01) after 45 minutes but in tail immersion method, the extract showed significant activity at all tested dose levels after 30 minutes interval.

Tambe DA *etal*⁵ (2010) studied Analgesic activity of *Caralluma adscendens* roxb. (aerial parts). Petroleum ether, Ethyl acetate and Methanolic extracts of *Caralluma adscendens* aerial parts at the doses of 100 and 200 mg/kg was evaluated for the Analgesic activity using the hot plate and acetic acid induced abdominal constrictions in mice. *Caralluma adscendens* aerial parts extract showed significant Analgesic properties in all the models studied.

R. Ramesh⁶ (2010) Studied Analgesic Effects of the Aqueous Extracts of *Ipomea pes-tigridis* in Albino Mice. The ethanolic extract of *Ipomoea pes-tigridis*, Family: (Convolvulaceae) was evaluated for analgesic property using plate reaction time. The preliminary studies show that the ethanolic leaf extract of *I. pes-tigridis* has significant analgesic activity.

Rudrappa JN and Mohmoud R⁷ (2010) studied Free radical scavenging activity of *Echinops echinatus* roxb Root. Extracts of *Echinops echinatus* Roxb, roots were evaluated for radical scavenging activities using different in vitro models like scavenging of 2, 2 diphenyl-1-picrylhydrazyl (DPPH) radical, nitric oxide radical and superoxide anion.

Leena S and Sitaram K⁸ (2010) discussed Traditional uses of plants as cooling agents by the tribal and traditional communities of dang region in Rajasthan, India. *Echinops echinatus* Roxb. paste smeared on soles and palms to treat heatstroke.

Tyagi SN *etal*⁹ (2010) studied *in vitro* Antioxidant Activity of Methanolic and Aqueous Extract of *Flacourtia indica* Merr. Total antioxidant capacity of extract was found to be 260 µg/ml and 180 µg/ml ascorbic acid for methanolic and aqueous extracts respectively. The results indicate that both the extracts clearly have strong antioxidant effects. The freshly prepared extracts were subjected to preliminary phytochemical screening test for various constituents.

Patil SM *etal*¹⁰ (2009) studied *in vitro* antioxidant activity of methanolic extract of stem bark of *Gmelina arborea* roxb. (verbenaceae). In this study, the antioxidant activity of methanolic extracts of stem bark of *Gmelina arborea* Roxb. (Verbenaceae) (MEGA) was studied using various *in vitro* assays. The antioxidant activity of MEGA was evaluated by using the free

radical scavenging activity assay (DPPH method), reducing power assay, nitric oxide scavenging activity, hydroxyl radical scavenging activity and H₂O₂ scavenging activity.

Maryam Z *etal*¹¹ (2009) studied *in vitro* antioxidant activity and total phenolic content of four indian medicinal plants. The percentage decrease of 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) standard solution was recorded maximum for *Hemidesmus indicus* (77.0%) followed by *Plumbago zeylanica* (73.41%), *Acorus calamus* (20.88%) and *Holarrhena antidysenterica* (20.06%) extracts at a concentration of 100 µg/ml. Phytochemical analysis revealed the presence of major phytochemicals like alkaloids, glycosides, phenolics and saponins. Moreover, total phenolics concentration equivalents to gallic acid was found in the range of 59.50 to 109.0 mg/g of plant extracts, which correlated with antioxidant activity.

Dharmendra D *etal*¹² (2009)) studied *In-vitro* antioxidant activity of the ethyl acetate extract of gum guggul (*Commiphora mukul*) Total antioxidant activity was determined by thiocyanate method, which was measured at 500 nm. tocopherol was used as standard, the standard, showed 76.38 % inhibition at 500 µg/ml concentration while *C.mukul* extract showed 51.16 % inhibition at the same concentration. In these testing, a significant correlation existed between concentrations of the extract and percentage inhibition of free radicals and reducing power.

Borikar VI *etal*¹³ (2009) Studied Analgesic activity of the stem bark of *Bauhinia racemosa* Lam in Rats. Aqueous extract of *Bauhinia racemosa* Lam stem bark @ 200mg/kg body weight produced significant analgesic activity whereas 100mg/kg dose did not produce significant results when Compared with control.

Adeolu AA *etal*¹⁴ (2009) Studied Anti-inflammatory and analgesic activities of the aqueous extracts of *Margaritaria discoidea* (Euphorbiaceae) stem bark in experimental animal models. The extract at 50, 100 and 200mg/kg body weight reduced significantly the formation of oedema induced by carrageenan and histamine. In the acetic acid-induced writhing model, the extract had a good analgesic effect characterized by a reduction in the number of writhes when compared to the control.

Lokesh TN¹⁵ (2009) studied Analgesic activity of aqueous and alcohol root extracts of *Pergularia daemia* (forsk.) chiov. In eddy's hot plate method the aqueous extract showed significant analgesic activity at the doses of 500 mg/kg (p<0.01) and 1000 mg/kg (p<0.001) and

alcohol extract showed significant analgesic activity at the doses of 500 and 1000 mg/kg ($p < 0.001$).

Bhaskar VH and Balakrishnan N¹⁶ (2009) studied Analgesic, anti-inflammatory and antipyretic activities of *Pergularia daemia* and *Carissa carandas*. The results of this study indicated that the ethanol and aqueous extracts from roots of *P. daemia* and *C. carandas* possess significant analgesic, anti-inflammatory and antipyretic activities in rodent models.

Sangita S *etal*¹⁷ (2009) Studied phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of *Phyla nodiflora* linn. The results indicate that methanol and aqueous extract at 500 mg/kg body weight shows a significant ($p < 0.05$) increase in the urine volume and electrolyte excretion ($p < 0.001$) when compared to control. Both the extracts show significant diuretic activity. From the present study it may be concluded that the constituents present in methanol and aqueous extracts may be responsible for diuretic activity.

Achinto S and Muniruddin A¹⁸ (2009) studied Analgesic and anti-inflammatory activities of the extract of *albizia lebbeck* in animal model. In rat paw edema model induced by carrageenan, the extract at the 400 mg/kg dose level showed 36.68% ($p < 0.001$) inhibition of edema volume at the end of 4h. In the acetic acid-induced writhing test, the extract at the 200 and 400 mg/kg dose level showed 39.9 % and 52.4 % inhibition of writhing, respectively.

Sandeep R *etal*¹⁹ (2009) studied Diuretic and laxative activity of ethanolic extract and its fractions of *Euphorbia Thymifolia* Linn. The extract was found to produce significant diuretic as well as laxative activity in dose dependant manner. Fractions of the extract potentiated the observed activities. The activities may be contributed to the phytoconstituents present.

Umang P *etal*²⁰ (2009) evaluated Diuretic Activity of Aqueous and Methanol Extracts of *Lepidium sativum* Garden Cress (Cruciferae) in Rats. Urine volume was significantly increased by the two doses of aqueous and methanol extracts in comparison to control group. While the excretion of sodium was also increased by both extracts, potassium excretion was only increased by the aqueous extract at a dose of 100 mg/kg. There was no significant change in the conductivity and pH of urine after administration of the *L. sativum* extracts.

Singh S *etal*²¹ (2006) isolated Flavonoids from *Echinops echinatus*. A new isoflavone glycoside, echinoside (7), together with 7-hydroxyisoflavone, kaempferol-4'-methylether, kaempferol-7-

methyl ether, myrecetin-3-O-alpha-L-rhamnoside, kaempferol and kaempferol-3-O-alpha-L-rhamnoside, has been isolated from the whole plant of *Echinops echinatus*.

Padashetty SA and Mishra SH²² (2005) described antifertility activity of *Echinops echinatus* roots on male rats. The present study was undertaken to evaluate the effect of terpenoidal fraction prepared from the petroleum ether extract of the roots of *Echinops echinatus* on male reproductive parameters.

Khan MA *etal*²³ (2000) discussed Ethnobotany and taxonomic studies of *Echinops echinatus* Roxb. (Untkatara) from Potohar region of Pakistan. The ethnobotanical information was verified by cross-checking with the people of different localities. The plant is diuretic, alterative, aphrodisiac and nervine tonic. It is also recommended in hysteria, dyspepsia, jaundice and scrofula.

Singh B *etal*²⁴ (1999) described anti-inflammatory activity of ethanol extract of *Echinops echinatus* whole plant. The extract effectively inhibited the acute inflammation induced in rats by carrageenan, formaldehyde and adjuvant and the chronic arthritis induced by formaldehyde and adjuvant.

Singh UP *etal*²⁵ (1998) investigated antifungal activity of some new flavones and flavone glycosides of *echinops echinatus*. Four phenolic compounds, viz., apigenin, apigenin-7-O-glucoside, echinacin, and echinaticin, were isolated from the whole plant of *Echinops echinatus* Roxb.; the latter two compounds were isolated for the first time. Echinacin, which was highly effective at 150 $\mu\text{g mL}^{-1}$, is considered the most promising of these compounds and its use as a control measure against *Alternaria* blight of pigeon pea under field conditions has been suggested.

Chaudhuri PK²⁶ (1997) isolated Echinozolinone, an alkaloid from *Echinops echinatus*. In addition to echinopsine and echinopsidine, a new alkaloid, echinozolinone, has been identified in *Echinops echinatus* as 3(2-hydroxyethyl)-4(3H)-quinazolinone from its spectral data.

Bupinder Sing *etal*²⁷ (1991) investigated antiinflammatory activity of taraxasterol acetate from *Echinops echinatus* in rats and mice. Taraxasterol acetate, a triterpenoid found in several plant species, demonstrated antiinflammatory activity in albino rats against carrageenan, formaldehyde and adjuvant induced inflammations in doses between 10 and 100 mg/kg.

3. OBJECTIVE OF WORK

- Medicinal plants are an integral part of human health care system. India is one of twelve leading bio-diversity centers with presence of over 45,000 different plant species. From this flora 15,000 to 20,000 have good medicinal value. Among those only about 7,000 plants are used in different system.
- The present century has many more health problem than earlier centuries. Drugs for diseases like AIDS, cancers, arthritis, and parkinsonism yet to be discovered. Recent survey revealed that more than 50% of all drugs issued by rational physicians are either directly derived from the natural source or synthesized by natural models as sole ingredient or as one of the several ingredients. Scientific study of medicinal plants offered a plethora of novel structurally diverse and bioactive compound. Multidisciplinary research on plants has lead to many drugs as well as prototype active molecules and biological tool.
- In present scenario of pharmaceuticals there is increasing demand for more active therapeutic agents. Apart from synthetic route, natural source is also a major source for the active therapeutic agent. There are number of medicinal plants in Ayurveda which required extensive study. The quality of herbs depends on various factors like cultivation, collection and storage conditions. Thus it is the need of the day to collect data for standardization and developed procedure based on macroscopic, organoleptic, microscopic, powder character, physicochemical constant as well as colored HPTLC chromatogram, finger prints and overlay spectra with marker compound for quick, correct and easy authentication and quality of herbal drugs. It will definitely help in enhancing the quality of herbal product and ultimately will build up the faith and belief of people in drug obtains from natural sources. Hence it is essential to establish herbal monograph and standardize the herbal drugs.
- The plant selected for herbal monograph was based on its availability and its various therapeutic activities in various ailments mentioned in Ayurveda. Literature review revealed that *Echinops echinatus* (Roxb.) are mentioned in Ayurveda but less work has

been carried out on them. This has inspired to set up the herbal monograph of *Echinops echinatus* (Roxb.).

- Aerial parts of the plant contain alkaloids, echinopsine, echinopsidine and echinozolinone. Taraxasterol acetate, isolated from the plant, is a potent anti-inflammatory constituent; the ethanolic extract of the whole plant is more effective when administered parenterally than orally. Apigenin and its derivatives, echinacin and echinaticin show antifungal activity.
- *Echinops echinatus* is bitter, stomachic, antipyretic, analgesic, increases the appetite stimulates the liver, useful in brain disease, used in ophthalmia, chronic fever, pains in the joints, inflammations and Urinary disorder.
- Main objective of this study is to prepare a monograph of *Echinops echinatus* that includes following parameters
 - There are number of species of *echinops echinatus* (Roxb.) present in nature so to identify *echinops echinatus* (Roxb.) from wild nature.
 - To evaluate Morphological and Microscopical characters of root, stem and leaf for further identification of plant.
 - Phytochemical investigation of root and aerial part by various chemical tests.
 - Various traditional books described that whole plant is medicinally used so there is required to find out either underground part or aerial part is more active for Antioxidant activity, Analgesic activity, Diuretic activity and Anti-inflammatory activity.

4. PLAN OF WORK AND METHODOLOGY

Plan of work

1. Collection & authentication of plants
2. Macroscopic evaluation
3. Microscopic evaluation
4. Determination of Ash value
5. Determination of extractive value
6. Successive solvent extraction
7. Preliminary phytochemical screening
8. Chromatographic studies: TLC and HPTLC
9. Determination of total Phenolic content
10. Determination of total Flavonoid content
11. *In vitro* Antioxidant activity of *echinops echinatus* (Roxb.) root and aerial part.
12. *In vivo* Analgesic activity of *echinops echinatus* (Roxb.) root and aerial part.
13. *In vivo* Diuretic activity of *echinops echinatus* (Roxb.) root and aerial part.
14. *In vivo* Anti-inflammatory activity of *echinops echinatus* (Roxb.) root and aerial part.

Methodology

Collection and Authentication of *Echinops echinatus* (Roxb.)

The whole plant material of *E. echinatus* is collected from wild nature and authenticated by Botanist.

Macroscopic evaluation

The morphology or macroscopical description of a crude drug include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc are carried out.

Microscopic evaluation

a) Transverse section of the Root, Stem and Leaf: Thin sections of the root, stem and leaf are taken and stained with phloroglucinol & hydrochloric acid (1:1). It was mounted in glycerin and observed under low and high power.

b) Powder microscopy: The coarse powder is boiled with chloral hydrate for 5 minutes, then stained with phloroglucinol and HCL (1:1) and observed for the microscopic features under high power (40 x).

Determination of Extractive values

Determination of various extractive values like Ethanol, Petroleum ether, Water and Chloroform are carried out by standard method described in Ayurvedic Pharmacopoea.

Determination of Ash values

Determination of Ash values like Total Ash, Water soluble Ash, Acid insoluble Ash and Sulphated Ash are carried out by standard method described in Ayurvedic Pharmacopoea.

Successive solvent extraction:

10g of the air-dried powdered plant material is successively extracted with the following solvents of increasing polarity in a soxhlet apparatus.

Petroleum ether (60° - 80°C), Benzene, chloroform, ethyl acetate, methanol and Water

Preliminary phytochemical screening

The presence of various phytoconstituents viz. steroids and terpenoids (Leibermann Burchard test), alkaloids (Dragendroffs test), tannins and phenolics (Ferric chloride test), flavonoids (Shinoda test), Sugars (Fehling solution test), amino acids (Ninhydrin test), etc. are detected by usual methods prescribed in standard texts.

Chromatographic study

TLC and HPTLC are carried out by using different solvents and find out number of important chemical present into it.

Determination of total Phenolic content and total Flavonoid content

Determination of total Phenolic content and total Flavonoid content by using appropriate method described into standard texts.

***In vitro* Anti oxidant activity**

Following different models are used for activity

- Ferric reducing antioxidant power (FRAP)
- DPPH radicals scavenging activity
- Scavenging of Hydrogen Peroxide
- Super oxide free radical scavenging activity

***In vivo* Anti inflammatory activity**

Anti-inflammatory activity of a compound is determined by Carageenan induced Rat paw oedema models.

***In vivo* Diuretic Activity**

The diuretic activity of Methanolic extract of Root, Methanolic extract of Aerial part and frusemide is carried out by using *in-vivo*, Lipschitz test method.

***In vivo* Analgesic activity**

The Analgesic activity of Methanolic extract of Root, Methanolic extract of Aerial part and standard drug is carried out by using Hot plate method, Tail immersion method and Tail flick method.