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Biosystematics studies on medicinal plant *Urginea indica* Kunth. liliaceae - A review

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Abstract

Indian squill, *Urginea spp.* is a very important and rare medicinal plant endemic to India, Africa and Meditteranian Regions. It has magical potential to heal many human diseases with cardiatonic, anticarcinomic, anti jaundice, anti dropsy, anti asthmatic, anti epileptic, dermatological and diuretic properties. Besides it has abortifacient effects and affects on menstrual cycle. It also finds its use as pesticides against fungus, insects and rats. Wide genetic and chromosomal variations were also still being researched to differentiate the different populations of *Urginea*. The biodiversity and germplasm collection is also a major area of emphasis to protect the rare genus. The basic taxonomic work to higher molecular developmental studies are still being explored in this genus. It is also a great source for many organic compounds yet to be characterized and discovered for its extensive possibility as potential bioactive molecule. The genetic variability and genomic studies are still being a hot topic in research.

Key words: *Urginea*, Medicinal plant, Phytochemicals, biodiversity, botanical pesticides

Introduction

The genus name *Urginea*, derived from an Arabian tribe, Ben Urginea was coined by a German botanist Adolphe Steinhill (1834) along with the identification of seven species[55]. Lindley (1836) placed this genus under the tribe Scilleae[33]. *Urginea* is one of the interesting and extremely polytypic genus with about hundreds of species occurring in India, Africa & Mediterranean region[4]. In Indian scenario nine species were seen most commonly under the genus Urginea[21]. Deb & dasgupta in a taxonomic revision recognized five species of the genus pertaining to India[13].

Urginea is commonly called as Indian squill finds its application in pharmaceuticals as well as in agriculture. Ancient Egyptians discovered its use against edema, emesis and cough. In modern medicine also it continues to find its use as an expectorant, with some commercial cold preparations. Ancient Romans used the extract of bulbs as cardiotonic is still being researched. Because of the popularity of the digitalis glycoside squill components, its use is restricted in United States as cardioactive agents, even after the approval by the German commission in 1985 as 'E' for cardiac in sufficiency. Some verities of squill have been known to be effective as rodenticides for more than hundred decades[7,23].

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The species of *Urginea*, especially *U. indica* is highly polymorphic with two distinct categories. The first category is very unique with underground bulbs producing inflorescence without vegetative leaves, immediately after the first shower followed by severe summer. The second category produces vegetative leaves along with the inflorescence axis soon after the first monsoon showers.

Classification

Division	–	Liliophyta
Class	–	Liliopsida
Order	–	Liliales
Tribe	–	Scilleae
Family	–	Liliaceae
Genus	–	*Urginea*

Egyptian Ebers Papyrus was the first textual record about the squill long back in 1500 B.C. It contains an ancient preparation using specified portions of squill to cure heart disease[30]. Ancient Greek scriptures also give evidence about the discovery of sea squill and its properties. Theophrastus a physician, botanists of ancient Greece and one time student of Aristotle (371 to 287 B.C) mentions in his writings how well it restores human health. Dioscorides' (Circa 40 to 90 AD) Encyclopedia, about medical substances dedicated a chapter exclusively to describe the diuretic, anti-jaundice, expectorant, anti-colic and anti-asthmatic

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properties of sea squill with the detailed medical preparations and prescriptions.

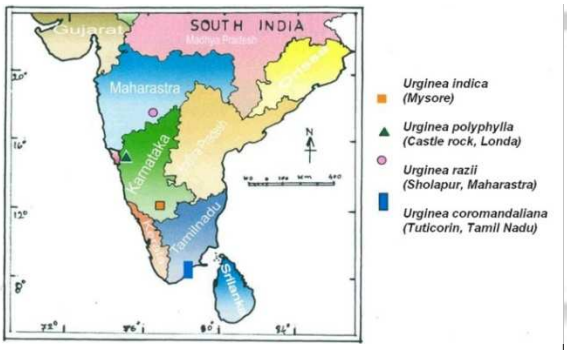
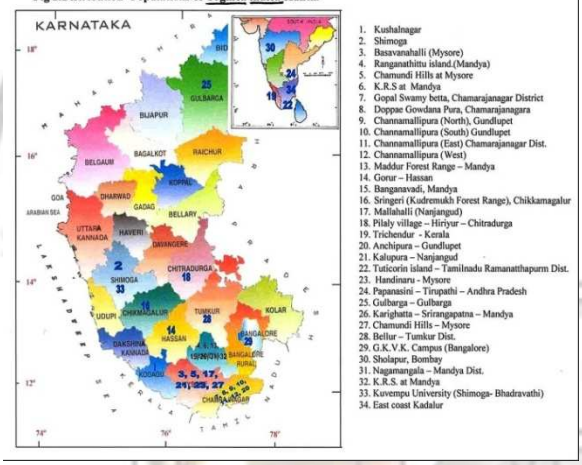


Fig 2.Distribution- Populations of Urginea indica Kunth.



It also referred squill as vegetable or salad with a note of caution about its poisonous nature. Pliny the Elder, the Roman author and naturalist (23 AD 79), categorized squill in to three types which included an edible one with a less pungent taste called 'Epimenides', describing the other two types by differentiating its appearance, taste and use as medicine. It was evident from fourteenth century documentations of Northern European countries about the use of squill vinegar prepared from dried squill. The Book of nature of Konrad of Megenberg, German scholar (1309 to 1374) referred a chapter on squill, describing it as mouse onion due to its rodenticide property. He identified edible squill varieties and also distinguished it as anti jaundice, anti dropsy and diuretic. Konrad of Megenberg also states that the Squill will cause abortion in pregnant women. The subject of the squill through history refer to the work of J. Stannard (1974), in ancient and medieval *Materia Medica* with special reference to its application for treating dropsy[53].

Language	Names
English	Sea Onion, Wild Onion, Indian squill, Red squill
Spanish	*Cebola Albarra*, *Cebola Chirle*, Esquila
Greek	Basal Fra aau, Basal El – Faar, Basal El – Onsol, Basal Nsool, Skeletoura
Hebrew	Hatsav Matsu
French	*Scille maritima*
Italin	*Cipolla Marina*
Portugese	*Cila Maritime*
Arabic	Feraoun
Hindi	Jangli Kanda, Jangli dungli
Tamil	Narivengayam, Kaattuvengayam
Kannada	Kadu Irruli, Vana Palandu, Kadu Bellulli, Naaiyiulli, Seeme nari Eerulli, Shiru Naarangaddhe
Synonyms	Basal tal ghansar, *Bulbo de escilla*, *Charybdis martima*, *Drimia maritime*, Ghansar, Meerzwiebel, Pharmacist's squill
New Names	*Cebolla Albarrana*, *Drima indica*, *D. Maritima*, European squill, Mediterranean squill, *Scilla maritime*, White squill, Sea onion, Sea squill bulb, *Urginea scilla*, *Urginea indica*, *Scilla indica*

Plant Description

Urginea indica Kunth. commonly called as Indian Squill is a perennial geophyte with fibrous roots of six to ten inches of length, proceeding from the base of the bulb is a scapigerous herb. The rounded conical, pear shaped bulbs with white transparent outer scales are about the size of an big onion, consisting of fleshy coats which are thin and papery red or orange brown in colour enclosing each other completely. The phyllotaxy exhibited is whorled hysteranthus or synanthus. The bulb, which is usually three fourth immersed in the sand sends several long linear lanceolate, radical, cauline, lorate, sessile, pointed and undulated shining, dark green leaves with a base sheathing, becomes two feet when fully grown. From the middle of the leaves, a round, smooth, long, terete, stiff and narrow succulent flower stem rises, one to three feet high terminating in a long, raceme, with close spike of whitish flowers, which stand on purplish peduncle. The flowers are bisexual, hypogynous, companulate bracteate and dropping. The flowers bloom in April and May after first shower followed by oblong capsules. The bracts are solitary, with long or short pedical. The perianth is lanceolate, subsequal in two whorls of three each outspreading, free to the base or very near to the base,

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one or few nerved at the centre. Stamens are six in number and are freely adherent to the base of the perianth segments. Anthers are ablong, dorsified, versatile in nature. Ovary is oblong or narrowly ovate, sessile superior, syncarpous, trilocular with short, thick and narrow style. It has subglobose stigma, with brownish capsule shaped shiny winged seeds, clustered, superposed and compressed in arrangements.

Vegetative Phase Reproductive Phase

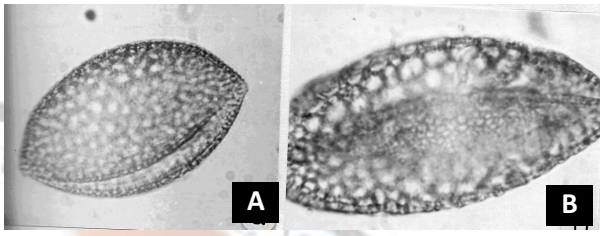
U. indica is a very good material for the study of the morphological variations has been recognized long back among the populations were not being used till date to differentiate it from other forms. This is probably due to the fact that no field collection ever contains both floral vegetative & reproductive phase features together. The predetermined parameters of different populations were assessed in two consecutive seasons to study and differentiate morphological variations from each other. At the population level the vegetative characters shown great variations on dependable taxonomic characters and mean time the reproductive characters shown insignificant uniform variations[52,37].

Floral Biology

The studies on floral biology in *Urginea indica* shows that the species of the present study are self incompatable and cross pollination is mostly by insects. Pollen fertility is observed as 82% with no seed setting. The data on the population studies have indicated that reproductive isolation through a difference in flowering period and blooming time as one of the factor that might have been played an important role in speciation and plausibly in the evolution of the genus.

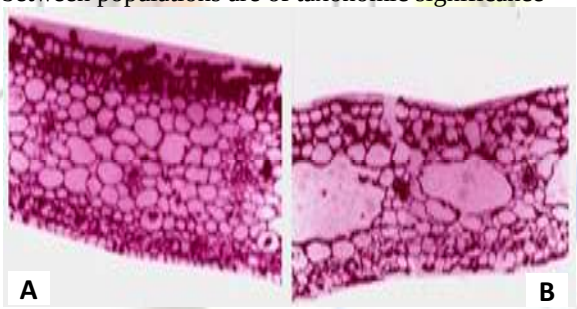
Pollen Grains[39]

Pollen grains are monosulcate oblong with a single colpae extending from one end to the another. But a careful analysis of pollen grains in populations of *U.indica* revealed finer differences especially with regard to the size and the quality of exine reticulation.

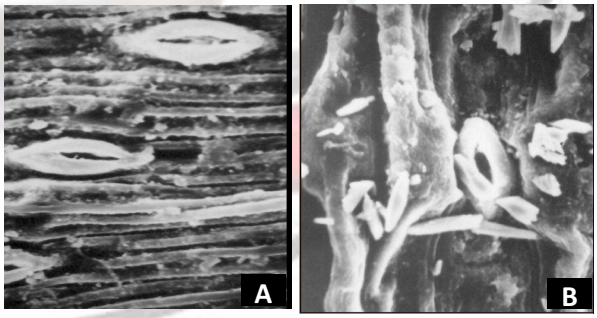


Anatomy [38,50]

Sixteen populations of *Urginea indica* (Kunth). Liliaceae were examined to provide the first detailed description of leaf anatomy following the methods employed by Johansen. The populations were distinguished into two types based on the fleshy and watery leaves. Populations vary with the features like presence or absence of thick cuticle, larger or smaller areoles and clear vascular bundles. The mesophyll cells with intercellular spaces filled with heavy and moderate wax deposition. Larger and smaller epidermal cells, palisade like tissue these characters along with other parameters plays an important role in delimiting the populations. Idioblastic cells containing raphide bundles with calcium oxalate crystals occur in the lower side of the mesophyll. These variations between populations are of taxonomic significance



Scanning electron microscopic studies of leaf surface



Leaf cuticular ornamentations stomatal variations and the differences in wax deposition, presence of raphides etc play an important role in delimiting the populations

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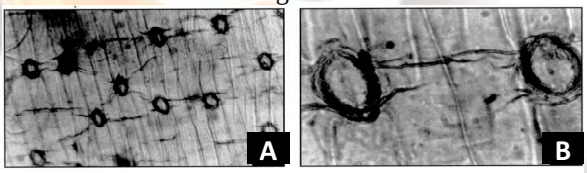
of *Urginea indica*. It is suggested that leaf surface characters can be used as secondary or supporting character in biosystema studies. Shiva Kameshwari et al. (2001)

Stomata

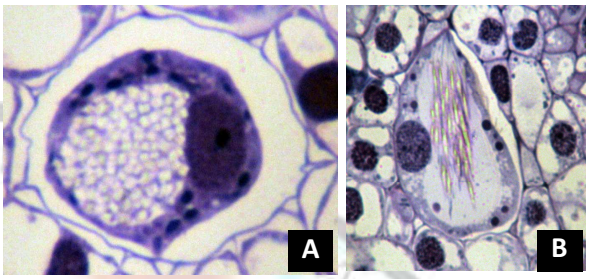
Studies were conducted on epidermal micromorphology of twenty one different populations of *U.indica* collected from various localities of Karnataka. They showed the presence both amphistomatic and amomocytic type of stomata on the upper and lower epidermis as reported as contributing variations in stomatal index. The comparative study on the matured leaves of different populations showed less variation in stomatal frequency, area of stomatal aperture and the size of guard cells [38].

Defense Role

Plant defend[1] themselves against attack from herbivores has been the subject of considerable interest over many decades. Plant structural traits such as raphides play an important role in protecting plants from herbivore attack. These raphides are evolved as a result of the response caused by the other environmental stimuli on the mesophyll interceullar spaces idioblastic cells containing raphide bundles and different phenotypes of crystalloid inclusions embedded in poly saccharides. Such crystals usually have backward oriented surface barbs capable of increasing damage to the mouth of grazing animals. Raphides are also responsible for producing mild inflammation and itching[12] when rubbed on the skin. Therefore, raphides take part in both mechanical and chemical irritation when they come into contact with tender tissues of soil living worms and herbivores[11].



The roots of *U. indica* play an important role in storing and utilizing water and nutrients thus protecting the plant from drought stress and environmental hazards. Raphides are present in all organs mean while bulbs showed richness in raphides. Thus calcium oxalate crystals occur in different forms in *U.indica* and perform various functions including herbivorous defense[25] calcium regulation (Volk et al., 2002) and are associated with heavy metal tolerance. (Franceschi and Nakata 2005)[50,52,37].



Distribution And Ecology

Sea squills with survive in areas with a little as 100 mm annual rainfall but are restricted to coastal regions. Quoting from a study by Kamal Hassan etal (1970) *U. maritima* is a polymorphic species with different varieties and forms Squill growing in Egypt show three distinctive features regarding the morphology of the bulbs. One with moderate size and reddish tinge, the second with white tunics and small size while the third with dark red tunics and very large bulbs[28]. This morphological variation is directly tied to soil type. In Egypt red bulbs inhabit soils of sand stone origin. Whereas white squill bulbs are found in soils of limestone origin. Whereas *U. indica* endemic to India, Africa and Mediterranean regions is found in a wide habitats ranging from desert, shrub, grassland, dunes and forests, soil conditions in which the squill is found are equally varied including sand, clay, calcareous, acidic and saline[37]. Recently it is found growing in platinum rich area Shiva Kameshwari and Paramasivam (2011) yet to be published

Germplasm

India being one of the natural centre of origin of *Urginea Indica* with large germplasm base, it is gaining immense global importance in view of its potential for multiple uses. Due to this, there is great scope & need to collect all species & their population & conserving them in one place.Therefore, there is immediate need to device a program aiming at systematic collection documentation & characterization of *Urginea* geruplasm in India. *Urginea* collections in India. And also a detailed germplam catalogue, comprising passport information on an the important attribute of different *Urginea* species including molecular ID cards is in progress.

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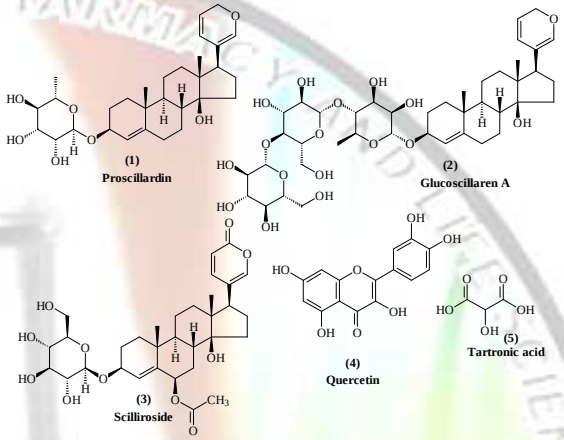
Production of Squill

White squill is a perennial herb native to the Mediterranean region, cultivated for drug extraction at Mediterranean regions, southern states of American and in the Caucasus regions. Turkey squill is harvested and traded locally from the wild area of different countries around the Mediterranean sea. Squill grows slowly and the bulb is not ready for harvesting until the sixth year when it produces its first lower stem. If it is allowed to continue growth then for several years the flowers can be harvested as cut flowers (Economic Botany). In India strict control of squill collection needs to be maintained in order to protect its wild populations. It is in progress in few hilly regions. For Eg: Siddarbhatta, Tumkur and Coimbatore, Wellingiri hills. Vegetative propagation was found to be stronger in desert populations of *U.undulata* compared with the Mediterranean populations of *U.maritima*.

Phytochemicals

Squill contains a large number of related steroidal cardioactive glycosides. Scillaren A and proscillaridin A, are the major glycosides found in the bulb in greatest concentration[59]. Other constituents found in squill include flavonoids, carbohydrates, antifungal glycoproteins, steroids, alkaloids, esters and saponins. Main active ingredients of squill are steroidal glycosides. Many new natural compounds have been isolated from *Urginea indica* by recent researchers are yet to be included in the organic chemicals repository[23].

The phytochemicals extracted from the bulbs were found to be potentially bioactive. 2, 3-Butanediol is used as a cardiac stimulant[8]. Along with these other compounds such as paraldehyde, tartronic acid, quercetin, and mindereru's spirit were also identified. Paraldehyde – a polymer of acetal dehyde is used as a sedative, hypnotic and anti seizure agent. It is a potent anticonvulsant capable of controlling seizures refractory to phenobarbital and phenytoin without causing respiratory depression[5]. Tartronic acid is used as an oxygen scavenger[3] in United States. Acid group shows the presence of mindereru's spirit which initiates perspirations.



Structures of some Bioactive Compounds

Steroids (corticosteroids) present in the bulbs were used to treat psoriasis by indigenous people. Quercetin, a potential bioactive molecule associated with wild onions were found significant in reducing the blood pressure by an average of five millimitres of mercury. Bufadienolides were identified in different chromosomal races of Indian squill *U.indica* (Shiva Kameshwari and Muniyamma ,2000). Identification of a novel 29 kDa glycoprotein with antifungal activity from Indian squill and its role in biological control were also being researched[31,35,46].

Table (1): The Important Compounds and the attached basic principal components responsible for bioactivity

SI.No	Activity	Compounds/ Basic Principal
1.	Acetylcholinergic	Glucose
2.	Anticarcinomic	Scillarenin
3.	Anticystic	Mannose
4.	Antidiabetic	Xylose
5.	Antiedemic	Glucose
6.	Antihepatotoxic	Glucose
7.	Antiketoic	Glucose
8.	Antirhinoviral	Scillarenin
9.	Antivaricose	Glucose
10.	Antiviral	Scillarenin
11.	Cancer preventive	Mucilage
12.	Cardiac	Scilliglaucoside
13.	Cardiotonic	Scillarenin
14.	Demulcent	Mucilage
15.	Diagnostic	Xylose

16.	Dye	Xylose
17.	Hypereglycemic	Glucose
18.	Memoryenhancer	Glucose
19.	Pesticide	Scillarenin

The leaf flavonoids in different populations of *Urginea indica* showed variations in the possession of cyonidin, petunidin & pelargonidin, they differ in their Rf values. Few populations are dissimilar since they are characterized by the absence of one of the three above mentioned compounds[54]

Pharmacology and Medicinal Uses[22]

Squill glycosides were identified for its cardiotonic properties similar to digitalis from Pharaonic times. However, squill components are less potent than digitalis. Preparations for oral administration are enteric coated to prevent degradation from gastric acid. Meproscillaren, a semisynthetic derivative of proscillaridin, is absorbed orally and may be effective in some patients. Based on British Pharmacopoeias assay for digitals there is no differences between extracts of *U. maritima* and *U. indica*.

Squill induces vomiting. Vomiting may be preceded by a generalized increase in the flow of secreations, and therefore these compounds appear to exert an expectorant effect in sub-emetic doses. Methanolic extract of red squill have been used as hair tonics in treating Seborrhea and dandruff [32]. Red squill is not used medicinally but used as rodenticides. Squill has been used traditionally as a cancer remedy and silliglaucosidin, has shown activity in an experimental, cancer cell line[16].

Squill is used in human homeopathy and phytotherapy and in veterinaryscience. It is administered orally, typically as a diuretic and functions by increasing blood flow through the kidney, emetic expectorant. In Greece fresh bulbs are distilled for medicinal use. The German commission E. Monographs suggests squill can be used for milder cases of heart insufficiency and also for diminished kidney capacity [European agency(1999),Blumenthal et al(1998)]. Sinistrin, an inulin like substance, is extracted from squill for use as a marker in diagnosis of renal problems.

Muscle pain from disease such as fibromyalgia, over work of a muscle which leads to cramps and contractures, contractures are continuous muscle contractions with associated chronic nagging pain. This type of chronic pain is difficult to treat. Use of squill extract for muscle pain has proved effective and developed as an analgesic[59].

Psoriasis an inflammatory disease of the skin, where treatments are not available in Allopathic medicine but *Urginea indica* preparations are known to be used traditionally by many tribes and aborigines against psoriasis and many dermatological diseases.

Cancer Activity

The activity studies conducted *in vivo* and *in vitro* against mouse mammary carcinoma cells proved that purified 29 kDa glycoprotein from squill as anticancerous. This protein assayed against cloned line of human colon adino carcinomatic $GC_3/C1^7$, KB Ch^r-8-5 and KB-3-1strain, using DMEM and RPMI media were also proved as anti cancerous.

Insecticidal Activity

The phytobiocative compounds extracted from *Urginea maritima* showed excellent mosquito repellent action[34]. Recent studies on *Urgenia indica* remarkably shown its larvicidal action against Aedes larvae causing dengue fever. The 400µl of aqueous lypholised extract showed cent percent mortality of the larvae within fifteen hours.

Toxicology and Agricultural Uses[22]

The Egyptian Alexandria plague epidemic of 1946 – 47, squill was used as rodenticide bait to control the spread of plague. Ab etel Gawad (1955) developed a Rat bait recepie using 1:3:1 proportion of white squill: flour: tallow with reasonable amount of salt by parted as one in three hundred portions, illustrates the potential toxicity caused by squill. Among the active principals, scillirosides shown greater bioactivity against rats exploiting the inability of rats to vomit the poison[20]. Because squill-laced bait is vomited by domestic animals before a lethal dose can be absorbed, often it is considered to be a rat-specific agent.

Christos Georgiades book 'Flowers of Cyprus' portrays about the potential possibilities of squill components as insecticides. Against storage pests of barley and wheat farmers normally use sea squill bulbs.

The larval growth retardant as well as adult fertility depressant actions of bufadienolids compounds extracted from bulbs of sea squill were found effective against storage pests like red flour bettle, *Tribolium castaneum*[34]. Recent research about the squill extracts leading to decrease the load of toxicants as synthetic pesticides and drugs or Neti merti (2008)Over dosage affects pregnant women and also plays its toxic role in menstrual cycle. The bulb extract showed hypoglycemic activity. The alcoholic extract is found active against *Entamoeba hystolytica* strain (C.P.Khare 2004) Rabbits, however, were found dead after they had chewed on fresh bulbs in the ground. Red squill a fine raticide, red squill plants should find a permanent home in the south west as a new specialty crop that should prove profitable to same farmers, increase the health of city dwellers and provide chicken farmers with better control of wasteful and pestiferous rats.

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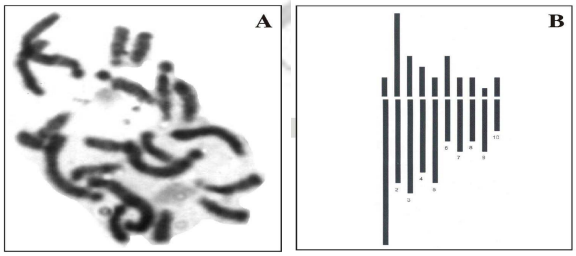
The studies on inhibitors in *U.indica* indicate their presence in leaves, bulbs and in seeds the concentration of the inhibitory substances in the bulb varies during its different growth phase[29].

Cytogenetics[36,40,41]



Recent attempts were made by researchers to distinguish *Urginea sps.* based on chromosome number and distribution to form an exclusive database through cytogenetic approach.

The general chromosome morphology of *U.indica* as a whole is quite distinct, the chromosome size ranges from very long to very short and often there is size difference in thirty two different population studies.

Chromosome number variability of *Urginea*

No	Species	Chromosome
1.	*U. altissima*	2n = 20
2.	*U. aurantiaca*	2n = 21
3	*U. burkei*	2n = 20
4.	*U. coromandeliana*	2n = 20
5.	*U. depreesa*	2n = 40
6.	*U. epigea*	2n = 32
7.	*U. fugax*	2n = 22
8.	*U. govindappae*	2n = 20
9.	*U. indica*	2n = 20
10.	*U. langii*	2n = 20
11.	*U. lydenburgensis*	2n = 32
12.	*U. maritime*	2n = 20
13.	*U. mouretic*	2n = 54
14.	*U. nigritiana*	2n = 60
15.	*U. polyphylla*	2n = 20
16.	*U. pretoreinsis*	2n = 20
17.	*U. rubella*	2n = 42
18.	*U. tenella*	2n = 20
19.	*U. undulata*	2n = 20

Cytological studies on populations of *U.indica* revealed the diploid, triploid, tetraploid, aneuploid and hexaploid nature of the populations through karyotype attributes using different parameters. (Shiva Kameshwari 1999)

Each cytotype studies differed distinctly in vegetative and floral characters. Consequently the thirty two cytotypes studied were observed by having distinct somatic complement of chromosomes. Thus, it lead into the recognition of different cytotypes among the natural populations. Several aneuploids have also been recorded in the recent investigations.

Polysomaty has been found to be a regular feature in plants which reproduce through vegetative means (Sen, 1973). The origin of such nuclei with varying chromosome numbers may involve various cytological mechanisms such as endomitosis, non disjunctions and duplication of chromosomes leading to polyploidy and aneuploid cells (Shiva Kameshwari and Muniyamma, 1999).

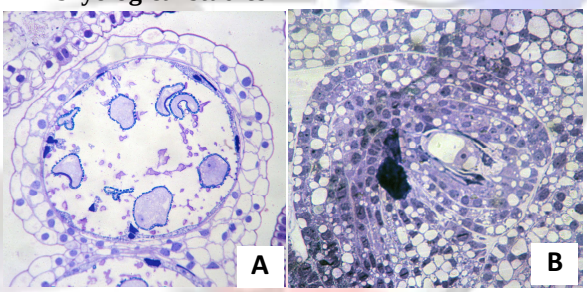
A team of researchers were still working on developing a cytological database on *Urginea indica* (Raghavan 1935). [41,42,46,47,51,52,61] Occurrence of cytomixis and its importance in evolutionary diversification of species were reported in diverse species of angiosperms (Shiva Kameshwari and Muniyamma, 2001, 2008). Meiotic irregularities & variations in the chromosomal behaviour indicates that the populations could eventually be treated as chromosome races.

B-chromosomes are also recorded during mitosis ranging from one to ten for *Urginea Indica.*

Karyotype

The karyotype in *Urginea indica* is asymmetrical with a graded series in which ST and SM type chromosomes predominate. It further suggests that the numerical evolution by polyploidy and structural changes leading to intra karyotypic size differences of chromosomes and shifting of centromeres from median to submedian and sub telocentric have been concomitantly operating in the genus.

Embryological studies



Microsporogenesis

The anther is four lobed with two middle layers. The endothecium shows evidences of disintegration as fibrous thickening in the inner middle layer. The tapetum is grandular with binucleate cells. Pollen grains are two celled. Microspore mother cells undergo reduction division resulting in the formation of isobilateral tetrads. Pollen grains show a smaller

generative cell and a larger vegetative cell. Pollen grains shows smooth, exine and thin intine.

Megasporogenesis

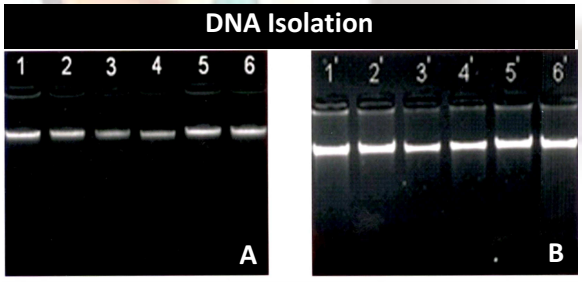
Megasporogenesis and the female gamet ophyte development conform the monosporic eight nucleate polygonum type of embryo sac development. However, the organized mature Embryo sac has a broader micropylar part and a narrower chalazal region in *Urginea indica*.

Plant Tissue Culture

Three chromosomal races (diplod, triploid and tetraploid) of Indian squill were screened for the production of bufadienolide proscillaridin-A (PsA) and scillaren-A(ScA) in tissue cultures. Bulbs and inflorescence segments were cultured on Murashige-Skoog medium supplemented with various combinations of plant growth factors, and /or coconut milk and yeast extract. Callus formation was induced from bulbs of diploid and triploid genotypes and from inflorescence segments of tetraploids. The shoot buds developed into small bulbous plantlets in plant PGF-free medium. Somatic embryogenesis was observed in the long-term callus cultures,. Shoot differentiating calli (callus with 10-20 shoot buds/8-12 weeks old) contains low levels of PsA in both the diplod, triploid and tetraploid races. All regenerated bulbous plants produced both PsA and ScA Jha (1991)[27].

Molecular Biology and Genetics

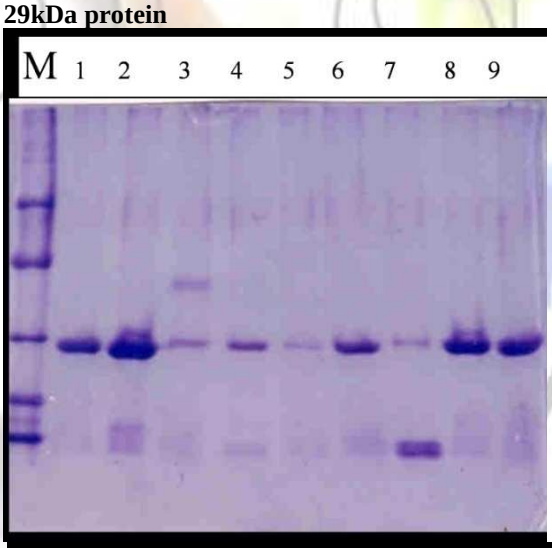
Molecular analysis primarily depends on the availability of high purity DNA sample and reproducible protocols for employed marker analysis. Here we present a first report on the optimization of DNA isolation and PCR conditions from bulb tissues of *Urginea indica*. The bulb tissues were used to study due to non-availability of leaf material during all the seasons. However, isolation of DNA from the storage tissue like bulb was particularly, challenging because of their high levels of polysaccharide and protein contents. Existing protocols described for other liliaceous species employed non bulbous materials; hence, were in effective for the present study. Therefore, several modifications were introduced to Doyle and Doyle (1987) method[15]. Finally to develop a protocol, which provided efficient in removing polysaccharides and proteins, thus yielding pure genomic DNA suitable for restriction digestion and PCR amplification. The extracted DNA showed characteristic restriction digestion pattern with four enzymes like Alul, Hind III, ECO RI and BAM H to standard DNA samples. The optimized, protocol for DNA isolation and PCR – RAPD analysis, standardized for the first time in *Urginea indica* using bulb tissue paved a way for future detailed molecular analysis in this important medicinal plant.



Urginea indica, being a plant with high therapeutic values, offers an excellent candidate for future medicinal applications. Analysis of the characteristics of different species and their populations at the molecular level may reveal genetic basis of variations in the plant properties, thus helping to study and improve these attributes in the plant. Marker systems such as RAPD, RFLP, SSR, AFLP etc., can be developed for the above stated purpose. In addition phylogenetic relationship offers an important tool for studying species relationship. Hence, there is an immediate need to undertake a detailed molecular characterization of *Urginea* species of India[15,54].

Biochemical Studies

29kDa protein



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An antifungal protein from *Urginea indica* bulbs was purified to homogeneity by acid precipitation, Diol-Gel filtration and C_{18} reverse phase HPLC. The molecular mass estimated as 29 kDa and periodic acid-Schiff (PAS) Staining showed that identified antifungal molecule is a glycoprotein. The neutralization of antifungal activity after periodic oxidation of 29 kDa glycoprotein suggests that the glycan part of the molecule appears to be involved in anti fungal activity. The N-terminal amino acid sequence of the purified protein responsible for the glycan part was determined as SQLKAXIXDF and had no sequence similarity with any other antifungal proteins. A polyclonal anti serum was raised against purified protein and used in immunolocalization analysis. *U.indica* protein exerts a fungistatic effect. It completely inhibits the germination of spores and hyphal growth of *Fusarium oxysporum*. The purified preparation contained a M_r 29kDa protein was observed as an active growth inhibitor of the fungal pathogens *Fusarium oxysporum* and *Rhizoctonia solani* in an *in vitro* assay. Amino acid sequence analysis of the M_r 29kDa protein revealed it to be highly homologus to the family 17 glycoside hydrolases, which are known to possess chitinase activity.

Urginea indica chitinase lacked a cysteine-rich N-terminal domain (characteristic of class I chitinase) and contained a conserved motif indicative of the signature 1 of family 19 glycoside hydrolases. It shared a ~70% sequence identity with the 26kDa endochitinase of *Hordeum vulgare*, a typical class II chitinase of family 19. The molecular weight, the lack of an N-terminal cysteine rich sequence, and the striking identity to the *H.vulgare* endochitinase suggest that the M_r 29kDa *U.indica* protein is a putative class II chitinase. The antifungal activity is presumably mediated through the chitinolytic activity of the M_r 29kDa protein[14].

Studies on Chitinase Protein in Urginea indica

The protein sequence given below is included in lysozyme like superfamily with specific hits for chitinase glycol hydro 19 protein having match with Chitinase in Barley, wheat, Rye, Maize, Garlic etc. (EC 3.2.1.14). It is also identified that the sequence had no identified sequence similarity with any other fungal proteins[30].

The FASTA file sequence is given below:

NH3-
SVSSIVSRAQAQPPKPSSHAFDRMLLHRNDGACQ
AKGFYTYDAFVAAAAAFSGFGTTGSADVQKREL
AQTSHETTGGWATAPDGAFAWGYCFKQERGAS
SDYCTPSAQWPCAPGKRYYGRGPIQLSHNYNYG
PAGRAIGVDLLANPDLVATDATVSADRAAGRVP
GFGVITNIINGGIECGHGQDS
-COOH

Percentage Contents of *Urginea indica*

SI No	Contents	Percent/100gms
1	Protein	8.36
2	Carbohydrate	66.25
3	Fats	0.32
4	Fibre	12.30
5	Moisture	6.16
6	Phosphorus	0.13
7	Acid value	7.23
8	Total ash	6.61
9	PH 5% solution	4.68
10	Fat	0.32
11	Energy	301.32 Kcal.
12	Calorific Value	3503 cal./gm

Microbiology

Indian squill *Urginea indica* is showing antifungal activity against *Fusarium oxysporum* which effects Nanjangud Rasabale, to *Sclerotium rolfsi, Alternaria tenuissima* and Rhizoctonia *solanii* which kills nursery plants[11].The extract was prepared with Methanol and are subjected for preliminary phytochemical and physicochemical analysis. The total ash content, acid insoluble and water soluble ash content were evaluated

along with the fluorescence characteristics of the methanolic extract of wild onion sps. The presence of primary and secondary metabolites such as *carbohydrates, proteins, alkaloids, phenolic compounds, saponins* were confirmed through preliminary phyto-chemical analysis.

The extract was found to possess anti-bacterial activity in *E. coli, S. aureus and P. aeruginosa* isolated from infected patients. The Minimum inhibitory concentration (MIC) was also evaluated by 'Tube dilution' method and the result was found to be considerably effective against selected pathogenic bacteria. Such an effect might contribute in explaining the traditional use of wild onion sps, *Urginea indica* in the treatment of wound healing.

The antioxidant activity was estimated by using DPPH free radical scavenging assay and the activity was increased with increase in concentration of methanolic fraction of wild Onion sps. The fractions of wild onion sps are free radical scavengers and are able to react with the DPPH radical, which might be attributed to their electron donating ability. Thus suggested the antioxidant components in this Wild Onion sps capable of reducing oxidants and scavenging free radicals. This also indicates that, tubers of wild onion, *Urginea indica*' are of therapeutic potential due to their high free radical scavenging activity. The presence of high amount of saponins justifies the practice of treatment for disturbances in the gastrointestinal tract by traditional healers.

The role of phyto-chemical constituents of this Wild Onion, *Urginea indica* sps in traditional medicine treatment was discussed. Hence, the formulation of extract of *Urginea indica* needs to be purified using biophysical techniques towards development of a potential drug/ lead molecule against microbial infection, inflammation and wound healing respectively.

Heavy Metal Analysis

No	Metals	Amount (mg/100gm)
1.	Silica	290.00
2.	Alumina	585.00
3	Iron	230.00
4.	Calcium	80.00
5.	Magnesium	22.00
6.	Sodium	65.00
7.	Potassium	120.00
8.	Copper	0.16
9.	Manganese	0.14
10.	Zinc	0.38

Conclusion

Urginea indica commonly called Indian squill is considered to have medicinal value and is largely used as an expectorant, cardiac stimulant, in treating rheumatism, dropsy, edema, gout, asthama and as an anticancer agent. Due to these properties, the squill bulbs have found place in British and European Pharmacopoeias. Squill bulbs have long been used as a source of natural product with Pharmaceutical and biocidal (rodenticide, insecticides and fungicide) application[34]. Due to unawareness we have lost many populations of *Urginea indica* and it is necessary to initiate awareness, conservation and cultivation of *U. indica*. Anthropogenic pressures such as habitat degradation are largely responsible for genetic depletion and loss of genetic diversity. New means and approaches are to be worked out for germplasm conservation and sustainable utilization of this economically important medicinal plant. The standardization of agro techniques and propagation program is in progress. Population studies have also been made in *U.indica*. An attempt has been made to enquire into the morphological variations which lead to evolutionary divergence of populations of *Urginea indica* Kunth. In particular, there were considerable morphological variations within the species. The thirty two cytotypes showed distinct morphological differences, in shape, size and colour of bulb and leaves, the length of inflorescence, and flower colour. The morphological complexity is accompanied by high degree of cytological variations. Preliminary measurement of reproductive characters have shown that no noteworthy results may be obtained in this characters except pedicel length but the vegetative character deviate significantly. The flowering and blooming time varied also played an important role in differentiating the populations. These morphological differences, have a genetic basis and would be worthy in recognizing them as a separate sub specific taxon.

Morphological and Cytological variations, revealed the presence of diploid, triploid, tetraploid, aneuploid and hexaploid populations and these were explored for their karyotype attributes using different parameters. We also report the presence of polygonum of eight celled embryosac development as well as *in vitro* culture and regeneration in one population. The bioactive principles of *U.indica* were extracted and tested for their antifungal and anti-cancer activities. The findings of the study indicated that the crude bulb extract can be used for various purposes. In the present study, we have isolated and studied the antifungal activity of a 29 kDa protein against, *Fusarium oxysporum* and *Rhizoctonia solani*. We have also identified the

antibodies developed against the protein which neutralizes its activity as fungicide. N-terminal amino acid sequence analysis showed that the purified antifungal compound protein is found to share high homology to 29 kDa Endochitinase group. *Hordeum vulgare* with a conserved domain Glyco hydro 19, characteristics of Chitinase Class I proteins. These data suggest that the purified antifungal compound is a Putative Endochitinase and could be a bonafide member of class II Chitinase showing anticancer activity. This investigation is aimed or making popular Indian squill an economic and medicinally important plant for India[35]

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