Phytochemical Screening, Antioxidant Activity and Flavonoids Analysis of Bulb Extracts of *Urgineaindica*Kunth

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Abstract

The Western Ghats of India are known to be a major biological hotspot that supports plant diversity and endemism. Members of the Liliaceae are famous for their use as medicinal herbs. *Urgineaindica*Kunth is glaborous, bulbus herb occurs in the forests of Maharashtra. The phytochemical study and antioxidant activity of the bulbs extracts of *Urgineaindica*Kunth were evaluated. Phytochemical screening indicated that, bulbs are rich in a variety of primary and secondary metabolites such as carbohydrates, alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides and saponins. HPTLC analytical method was developed for the chemical fingerprinting of *Urgineaindica*Kunth flavonoids. The method was validated in terms of their linearity, LOD, LOQ, precision and accuracy and compared with RP-HPLC-DAD method. Micro nutrients like Zn,Fe,Cu,Mn and Se were detected on ICP(Induction Coupled Plasma).Our research highlights the biochemical and ethno pharmacological significance of *Urgineaindica*Kunth.

KEYWORDS:*Urgineaindica*Kunth, Phytochemicals, Antioxidants, Flavonoids, Medicinal plants

INTRODUCTION

*Urgineaindica*Kunth, the"Indian squill", a perennial glabrous herb belongs to family liliaceae,is a commonly known as "Junglipiyaz"in Pakistan, It grows in Salt Range, Kotli Near Mirpur and Mt.Tilla (Baquar, 1989). In the indigenous traditional system of medicine, bulbs or rhizomes of *U. indica*possess several therapeutic significance, in chronic bronchitis, deobstruent, digestive, expectorant, stomachic, diuretic, emmenagogue, purgative, hypoglycaemic, anticancer activityand asthma. The other actions attributed to *U. indica*are anthelmintic, cardio-tonic in heart insufficiency, use in calculous and paralytic affections, rheumatism, leprosy, skin diseases, internal pain and scabies etc. (Baquar, 1989;Kirtikar and Basu, 1988; Prajapati*et al.*, 2003). Pharmacological evaluations have revealed the presence of antibacterial, antifungal (Shenoy*et al*.,2006), laxative and spasmodic (Abbas *et al.*, 2012),antioxidant, anti angiogenic and pro-apoptotic activities in *U. indica*(Deepak and Salimath, 2006).Crushed or sliced bulbs are also applied at feet sole to prevent burning sensation (Kapoor, 1990; Usmanghani*et al*., 1997). However, externally used for removing corns and warts (Kapoor, 1990; Prajapati*etal*.,2003).Wild onions tend to develop small bulbs with shallow roots used to cure infectious wound (Benkeblia,2004).Dry skin of wild onion is

used as a yellow dye, it contains Quercetrin which is anti-allergic and is also helpful in treating inflammatory bowel disease (Brodnitz*etal*, 1971).

Despite of its extensive medicinal application in airways hyperactivity disorders and also in cardiac disorders, *U. indica*has not been studied widely to evaluate its medicinal uses.Among phytochemical constituents, the glycosides,scillarin-A and scillarin-B have been adequately found in fresh squill (Prajapati et al., 2003). Other constituents observed in squill include flavonoids, carbohydrates, antifungal glycoproteins, steroids, alkaloids, tannins, coumarins and saponins (Abbas *etal*., 2012; Kameshwari*et al*., 2012).

In traditional medicines, medicinal plants have contributed hugely to the traditional and western medicines through providing ingredients for drugs or having played central roles in the drug discovery. The evaluation of a crude drug is an integral part of establishing its correct identity. Before any crude drug can be included in herbal pharmacopoeia, pharmacolognostical parameters and standards must be used for assessment of quality consistency and stability of herbal extracts or products by visible observation and comparison of the standardized fingerprint pattern (Rajkumar,*et al.*, 2010). Herein, we reported the phytochemical analysis, the chemical fingerprint pattern of flavonoids by HPTLC method and antioxidant activity of *Urgineaindica*Kunth bulbs.

Fig.1:Habit of *Urgineaindica*(BX4)Fig. fromKarjat.

2:Habit of *Urgineaindica* (BX5)from Kalbadevi.

Fig.3:Bulb of *Urgineaindica*(BX4*)* from Karjat

Fig. 4:Bulbs of *Urgineaindica*(BX5) from Kalbadevi.

2. MATERIALS AND METHODS

2.1 Chemicals

All solvents were distilled prior to use. TLC was performed on silica gel 60 F254 (Merck). All reagents and solvents purchased from Merck Chemicals. Minerals detection was performed by using CEM Mars 6 microwave digester and Teledyne Leeman, ICP OES model Prodigy Dual View (Induction Coupled Plasma).The HPTLC were recorded on CAMAG HPTLC system (Switzerland).

2.2 Sampling

Fresh samples of bulbs of *Urgineaindica*Kunthwere collected during monsoon (June 2012 to September 2012) from Kondhane village,Karjat and Kalbadevi village,Ratanagiri regions of Western Ghats of Maharashtra (Figures 1,2,3 and 4). These plants were identified and authenticated using herbarium collection at Botany Research Laboratory, DST-FIST School of Life Science,SRTM University,Nanded(MS).Fresh bulbs were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder (Panasonic make). This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature to carry out phytochemical screening of secondary metabolites.

2.3 Soxhlet Extraction

Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with accurately weighed 10 g of the drug powder for 18-40 h. Extraction was performed with water, methanol, chloroform, acetone and IPA as the extracting solvent. The extraction was conducted for 6-8 h/day and finally all the extracts were evaporated under vacuum.

The water, methanol, chloroform, acetone and IPA extracts of bulbs of these plants were prepared according to standard methods (Harbone, 1998). Nitrogen gas was purged through these extracts to prevent oxidation of secondary metabolites. These extracts were sealed in airtight containers and stored at -4^0C.

2.4 Phytochemical Screening: Phytochemical screening of active plant extracts was done by following the standard methods for the qualitative analysis of various phytochemical studies such as alkaloids, carbohydrate,glycosides, saponins, flavonoids and phenols which could be responsible for antioxidant activity (Table 1).

2.4.1Antioxidant activity: DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.0394 gm DPPH in 1000 ml methanol. The solution was kept in darkness for 30 minutes to complete the reaction. The free radicals scavenging activity of the crude extracts was determined by the 1,1-diphenyl-2-picryl-hydrazil (DPPH). The antioxidant activity was measured by the standard method(Brand-Williams *etal*,.1995). Wherein the bleaching rate of stable free radical, DPPH was monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 570 nm, but upon reduction by an antioxidant or radical species its absorption decreased. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = (ABS $_{control}$- ABS $_{sample}$)/(ABS $_{control}$)X 100), whereasABS $_{control}$is absorbance of negative control and ABS $_{sample}$ is the absorbance of the reaction mixture containing the sample extract.

2.4.2 Mineral analysis

Micro-scaled digestion:

CEM-MARS 6 microwave oven was used for micro-scaled digestion. 0.5 gms of herbal samples were weighed and transferred to CEM- Xpress vessels. 8-10ml of conc. HNO_3 was added to the samples. The samples were predigested for 10-15 minutes prior to capping the vessels. The CEM- Xpress vessels were assembled for microwave irradiation. The microwave program was adjusted with respect to the number of vessels and reference to the guidelines of CEM at 1000W with 100% level. A 25 minutes ramping period was used to reach the digestion temperature of 180^0C which there upon was maintained for 15 minutes. The CEM- Xpress vessels were kept in fume hood for cooling and to release the pressure by uncapping. The contents were transferred to 50 ml volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use.

Calibration Standards:

For calibration, Leeman and Thomas Baker Std. sample were used as the reference for the calibration range.

Instrument Preparation/Operation:

The spray chamber, nebulizer & torch assembly was completely cleaned to eliminate any form of contamination. The plasma was stabilized for 15 minutes by flushing with distilled water. An Instrument Calibration was performed to check the wavelength shift and the same was successful with a minimum deviation of <10 % with master scan.

ICP mineral analysis: Diluted samples were used for further analysis by using Teledyne Leeman, ICP (Induction Coupled Plasma).

2.4.3. Flavonoids analysis by HPTLC

Standard preparation

A standard Quercetin, Kaempferol, Hesperdin,Catecingallate and Rutin manufactured by SIGMA Aldeich (USA) were used.10mg ofQuercetin in 5mL ethanol,20 mg of Kampherol in 1mLeyhano, 5 mg ofHesperdin in 5 mL water,1mg of Catecingallate in 0.25 mL methanol and 250 mg Rutin in 5 mL pyridine were dissolved. The pre-treated sample extracts and stock solutions were filtered through 0.45- µm syringe filters.

HPTLC method

HPTLC analysis was carried out by reported method (Harborne, 1973and Wagner *et al.*, 1996).We have used CAMAG HPTLC system equipped with Linomat V applicator, Thin-Layer Chromatography (TLC) scanner 3,Peprostar 3 with 18.2 Mega pixels CCD camera for photo documentation , controlled by Win CATS -4 software.The samples (10 µL) were spotted in the form of bands of width 5mm with a Camagmicro litre syringe on

silica gel 60 F254 (20 cm X 10 cm with250 µm thickness) plates (Merck) using a CamagLinomatV (Switzerland).The plate loaded with samples was kept in TLC twin trough developing chamber (after saturated with solvent vapour) with respective mobile phase optimized for flavonoids. The plate was developed in the solvent system with ethyl acetate, formic acid, acetic acid, water at the ratio (25:2.7:2.7:6.9) up to 90 mm.Linear ascending development was carried out in 20cm X 10cm twin trough glass chamber (Camag,Mutenz, Switzerland).The chromatoplatesaturated with mobile phase was kept twice in andthe same mobile phase for good resolution of chromatogram of chemical fingerprinting.The optimized chamber saturation time for mobile phase was 30 min at room temperature $(25\pm2)^0$C. The developed plate was dried by hot air to evaporate solvents from the plate.The developed plate was sprayed with ice coldsolvent system of sulphuric acid and methanol (20:180)and dried at 100^0C on digital hotplate for 2 min.The plate was photo documented at UV 254 nm,366 nm and day light using photo–documentation (CamagReprostar 3) chamber.The plate was fixed in scanner stage and ultimately, scanning was done at 366 nm. Further, the plate was kept in photo documentation chamber (CamagReprostar 3) to capture the images under White light,UV light at 254 nm and 366nm respectively.Densitometric scanning was performed on Camag TLC scanner III which was operated by CATS software.

3. Result and Discussion:

3.1. Optimisation of extraction method

In order to extract the phytochemicals from herbal samples efficiently,variables involved in this procedure were optimised,including extraction solvent (Water, Methanol, Chloroform, Acetone, IPA, 100%), extraction method (Soxhlet, reflux, percolation), and extraction time (18-40 hr). The extraction time in water was 40 hr. The biomass was refluxed for 40hrs,and then it was dried naturally for 2-3 days. To the dried biomass, 100% methanol was added and the reaction was percolated for phytochemicals. The methanolic fraction was collected in amber coloured bottle under nitrogen atmosphere. The material was dried for 5-6 hrs. The procedure was repeated for chloroform and acetone. The extraction time was optimized for all the samples. All the extracts were preserved under nitrogen atmosphere in amber coloured bottle.

3.2. Phytochemical Screening

It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils. The phytochemical profiling is necessary for local medicinal plants usually employed by herbalists in the treatment of diseases(Banso and Adeyemo, 2007).The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. For example, saponins are a special class of glycosides which have soapy characteristics and havebeen reported to be active antifungal agents. Antimicrobial properties of a number of tannins, flavonoids, alkaloids have been reported. Not only the antimicrobial properties have been ascribed to these plant phytochemicals, but other biological activities including modulation of the immune system have been assigned to these compounds in plants.

Phytochemical screening of the bulbs extracts of *Urgineaindica*revealed the presence of different phytochemicals. Indeed phytochemical investigations of this plant have resulted in occurrences of carbohydrates, alkaloids, glycosides, saponins, flavanoids, phenols, Vitamin E and Vitamin C. Table 1 illustrates the results of phytochemical screening of all the extracts of *Urgineaindica*. The qualitative analysis of carbohydrates(Benedict's reagent test)and glycosides (Borntranger's Reagent) were carried out in all extracts i.e. aqueous (S1), methanol (S2), acetone (S3) and chloroform (S4) extracts. The solutions turned red and pink confirmed the presence of carbohydrates and glycosides respectively. The hydrophilic carbohydrates and glycosides were present in water (S1) whereas hydrophobic carbohydrates and glycosides were detected in rest of the organic solvents (S2-S4). The Mayer's test of extract S2 displayed appearance of white turbidity for alkaloids. The alkaloids were absent in S1, S3, S4, extracts.The dark brown coloration test for phenols was observed in S2-S4extracts. The water soluble phenols were absent in all the extracts. The extracts S1-S4 were shaken with distilled water. The persistence of froth in S1, S2 was observed, indicated the presence of saponins. The hydrophilic flavonoids were detected in extract S1. The water soluble vitamin C was found in S1and the vitamin E was qualitatively analyzed by HPLC method in extracts S3 of *Urgineaindica.*

Table 1: Preliminary phytochemical screening of bulbs extracts of *Urgineaindica.*

Constituents	**Test**	**Observation**	**Plants**							
			***Urgineaindica*(BX4)**				***Urgineaindica*(BX5)**			
			S1 W	***S2 M***	***S3 C***	***S4 A***	***S1 W***	***S2 M***	***S3 C***	***S4 A***
Carbohydrates	Benedict's Reagent	Red precipitate	+	+	+	+	+	+	+	+
Alkaloids	Mayer's Reagent	White precipitate	-	+	-	-	-	+	-	-
Glycosides	Borntranger's Reagent	Pink coloration	+	+	+	+	+	+	+	+
Saponins	Foaming	Frothing persisted for 10-15 min	+	+	-	-	+	+	-	-
Flavonoids	Shinoda	Pink-Red colouration	+	-	-	-	-	+	-	-
Phenols	Ferric chloride	Dark brown coloration	-	+	+	+	-	+	+	+

Vitamin C	2,6-dichlorophenol-indophenol sodium salt	Red Coloration	+	-	-	-	+	-	-	-

S1=Water, S2=Methanol, S3=Acetone, S4=Chloroform.

3.3Antiscavaging activity:

The phytochemical screening of the crude bulb extracts showed the positive reactions for alkaloids,flavonoids,phenols,saponins, glycosides, carbohydrates, Vitamin C, Vitamin E and minerals. The scavenging ability assayed is the ability of extracts to react rapidly with DPPH radicals and reduce most DPPH radical molecules. The antioxidant capacity *Urgineaindica* bulbs extracts was measured by DPPH antiscavenging activity method and the results were expressed in table 2. The DPPH antiscavenging activity of aqueous extract was 62.41%in BX4 and 30.66 in BX5; higher than those of methanolic extract of BX4 and 4 folds higher than chloroform and acetone extracts. (Table 2) However, the DPPH antiscavenging values of methanolic extract (71.54%) in BX5 and water extract of BX4(62.41%) were for comparable. The methanolic extract of BX5 displayed significant antioxidant activity. The results obtained from various observations suggested that the alcoholic extracts have higher potential inmedicinal suitability as antioxidantagents.

Table 2:Antioxidant Activity

Sr.no.	**Species Name**	**Code**	**Extract**	**Anti-Scavenging(DPPH) Activity (%)**
1	***Urgineaindica***	**BX-4**	Water	62.41
			Methanol	38.37
			Chloroform	19.82
			Acetone	16.69
2	***Urgineaindica***	**BX-5**	Water	30.66
			Methanol	71.54
			Chloroform	20.89
			Acetone	25.53

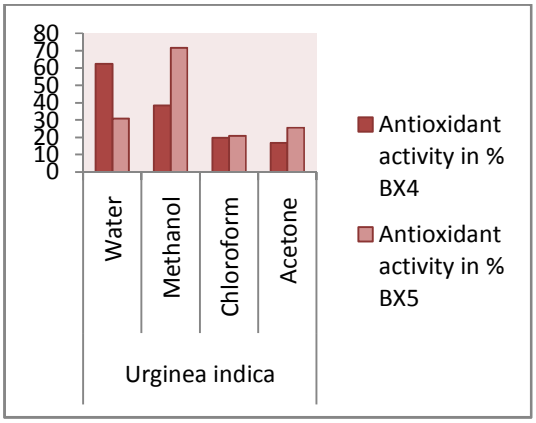


Fig 5: DPPH antiscavenging % in bulb extractsof *Urgineaindica*

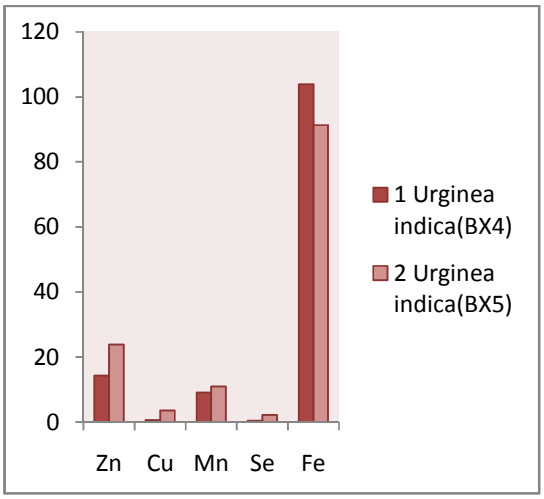


Fig. 6:ICP mineral analysis in bulb of *Urgineaindica.*

3.4 Mineral analysis:Optimization and calibration for of*Urgineaindica*bulb extracts

After optimization, a new calibration method was developed for these samples.The wavelengths used for calibration were Cu 324.754 nm, Mn 257.610, Se 196.090, Fe 259.940, and Zn 213.856 (Table 3). Calibration standard solutions were measured 3 times one by one with an RSD < 1%. After calibration with standard solution, a necessary back ground correction was applied for each wavelength. The samples were measured thereafter with 3 cycles. The average sums of the 3 measurements weretabulated in the analysis report.

Quantitative multi-elemental analysis by inductively coupled plasma (ICP) spectrometry depends on a complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task which constitutes a bottleneck in modern multi elemental analysis. Additional obstacles may be that sample quantities are limited and elemental concentrations low. In such cases, digestion in small volumes with minimum dilution and contamination is required in order to obtain high accuracy data.

We have developed a micro-scaled microwave digestion procedure and optimized it for accurate elemental profiling of plant materials. A commercially available 40- position rotor with 5 mL Polytetra flouro ethylene (PTFE) vials, originally designed for microwave-based parallel organic synthesis, was used as a platform for the digestion. The novel micro-scaled method was successfully validated by the use of various certified reference materials (CRM). The micro-scaled digestion procedure was applied on crude powder of dried plant material in small batches. The contents were transferred to 50 mL volumetric flasks and volumes were made with distilled water. The solutions were filtered prior to use. Teledyne Leeman, ICP spectrometer was calibrated by using Leeman standard, National Institute of Standards and Technology (NIST), USA. Diluted samples were used for further analysis.

Iron and copper are of great importance for life. As redox-active metal they are involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, hormone biosynthesis.Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of biomembranes. Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing, and translation. Although the essentiality of Se to plants has not been established yet, Se is considered a beneficial element in promoting plant growth in some plant species.

We have determined the 5 elements in coarse powder of bulbs of *Urgineaspecies*(Table 4). Thereby, the concentration of minerals in bulbs extracts had the different profiles in both *Urginea species* and quantitative differences had been detected.

The most abundant microelement was Fe in *Urgineaspecies*; whereas copper was found at the lowest concentration in both extracts. The content of Iron was especially high in comparison to Zn, Cu, Mn and Selenium.The concentration of Zn content was comparable in both the species.Diatary antioxidants include selenium,vit.A and the related carotenoids,vit.C,vit.E(Devare*etal.,* 2011).Selenium is recommended to increase the number of large bulbs and increase bulb antioxidant capacity(Poldama*etal.,* 2011). Presence of selenium in bulbs shows a strong antioxidant potential.

Table 3: Instrumental characteristics and setting for ICP-OES:Spectrometer LEEMAN LAB's Simultaneous ICP-OES PRODIGY XPDualSystem

	Parameters Range		Actual Parameters
	Min	Max	
Power	0.1	2.0	1.1 KW
Coolant Flow	5	20	18 L/Min
Auxiliary Flow	0.0	2.0	0.2 L/M
Nebulizer Flow	5	60	34 psi
Plasma Torch	--	--	Dual

Spray Chamber	--	--	Cyclonic
Nebulizer	--	--	Concentric
Sample Aspiration Rate	0.5	2.0	1.4mL/min
Replicate read time	--	--	40 sec per replicate for Axial

Table: 4. Accuracy of elemental concentrations in *Urgineaindica*after micro-scaled digestion expressed in ppm

Sr.no.	Name of the plant	Zn	Cu	Mn	Se	Fe
1	*Urgineaindica*(BX4)	14.2622	0.6038	9.1262	0.369	103.8988
2	*Urgineaindica*(BX5)	23.8256	3.5554	10.9436	2.1622	91.3855

3.5 Flavonoids analysis by HPTLC

Flavonoids are ubiquitous in photosysnthesis and therefore occur widely in plant kingdom(Deshmukh, 2008).They are found in fruits, vegetables, nuts,seeds, stems, and roots and constituents of the human diet.The bulbs of *Urgineaindica* contains sulphur compounds, carbohydrates, proteins, phenolic compounds, saponins, quercetin (Kim, 1997).

The results of present study confirmed the presence of flavonoids in the aqueous,chloroform and methanolic extracts bulb of *Urgineaindica*.The results depicted in table 1for preliminary phytochemical screenings suggested the presence of flavonoids, steroids, alkaloids,glycosides, terpenoids, sugars and amino acids in the aqueous and methanolic extracts of*Urgineaindica*bulbs.The solvent systems of various compositions were used as mobilephase for the optimization of HPTLC analysis to obtain highresolution and reproducible peaks. The optimized solvent system, ethyl acetate-formic acid-acetic acid-water(25:2.7:2.7:6.9) was selected as the mobile phase (Table 5 - 10); (Figure 7. A-E);(Figue8. A - E). The aqueous extract of bulbof *Urgineaindica* (BX4) showed the presence of 6different spots of flavonoids having Rf values ranging from of 0.12to 0.91. In case of BX5, 14 different spots of variousflavonoids were observed havingRf values in the range of 0.13 to 0.98.

The methanolic extract of bulb of *Urgineaindica*(BX4) showed the presence of 7 different types of flavonoids with 7 different Rf values with range of 0.05 to 1.00 and the presence of 10different types of flavonoids with 10 different Rf values with range 0.12 to 0.86 in bulb of BX5. The chloroform extract of bulb of *Urgineaindica* (BX4) and (BX5)showed the presence of 3different types of flavonoids with 3 different Rf values with range 0.03 to 0.94 and 0.00 to 0.97 (Table.5-10). All values were compared with standard flavonoids(Fig.9)

Additionally, the chromatographic plate was scanned at various wavelengths (Figures 7-8). In figure 7-E, the chemical constituents were significantly separated at white light AD for BX-4. Similarly, in case of BX-5, the maximum spots were observed in figure 8-G. The blurred images suggested that the visualization of chemical fingerprint was not possible in UV region of wavelength 366-254nm.

Our results showed that the presence of Rutin in aqueous extracts of both the species.Quercetin in aqueous and chloroform extracts of BX5.Kampherol in chloroform extracts of BX4 and methanolic extract of BX5.

Table 5: HPTLC –Flavonoids profile of the aqueous extracts of *Urgineaindica*(BX4)– bulb.

Peak	Rf	Height	Area	Assigned substance
1	0.12	10.1	20454.2	Unknown
2	0.39	11.5	2019.3	Unknown
3	0.55	17.9	453.9	Rutin
4	0.62	6.0	647.0	Unknown
5	0.81	8.7	632.7	Unknown
6	0.91	8.7	349.8	Unknown

Table :6 HPTLC –Flavonoids profile of the aqueous extracts of *Urgineaindica*(BX5)–bulb.

Peak	Rf	Height	Area	Assigned substance
1	0.13	241.6	42089.1	Unknown
2	0.19	78.5	7286.5	Unknown
3	0.27	86.4	3962.6	Unknown
4	0.30	76.0	1566.7	Unknown
5	0.33	74.5	1531.5	Unknown
6	0.35	48.9	999.4	Unknown
7	0.41	55.3	2429.1	Unknown
8	0.49	125.2	6350.5	Unknown
9	0.55	57.9	4935.5	Rutin
10	0.65	47.4	4919.3	Hesperdin
11	0.69	14.4	1050.1	Unknown
12	0.76	7.6	594.6	Unknown

13	0.96	12.9	292.2	Quercetin
14	0.98	8.8	163.4	Unknown

Table:7 HPTLC –Flavonoids profile of the chloroform extracts of*Urgineaindica*(BX4)–bulb.

Peak	Rf	Height	Area	Assigned substance
1	0.03	13.5	301.0	Unknown
2	0.14	1.8	1490.8	Unknown
3	0.94	0.0	59.2	Kaempferol

Table:8 HPTLC –Flavonoids profile of the chloroform extracts of*Urgineaindica*(BX5) bulbs.

Peak	Rf	Height	Area	Assigned substance
1	0.00	5.5	128.2	Unknown
2	0.09	0.3	265.1	Unknown
3	0.97	1.2	97.6	Quercetin

Table:9 HPTLC –Flavonoids profile of the methanolic extracts of*Urgineaindica*(BX4)– bulb.

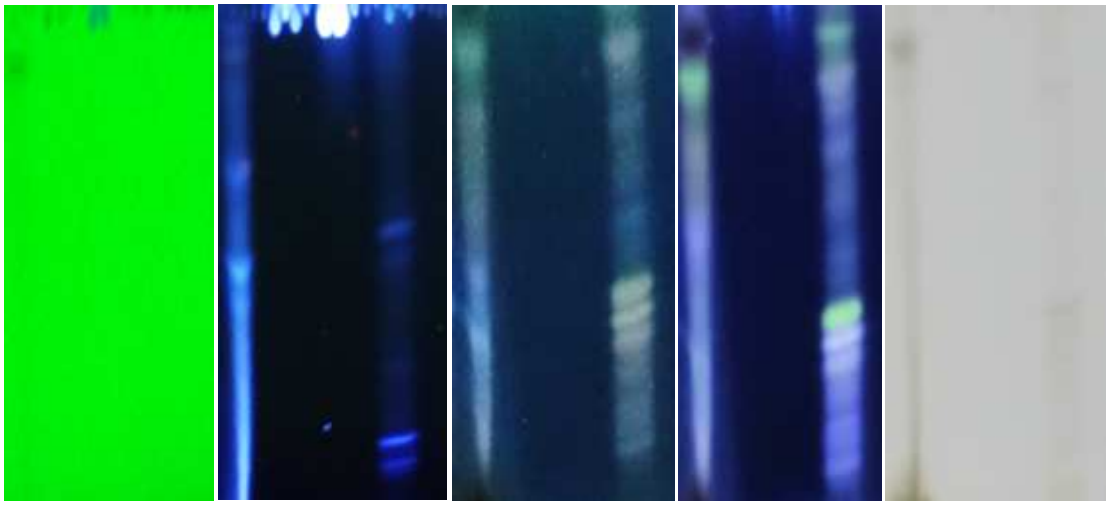
Peak	Rf	Height	Area	Assigned substance
1	0.05	8.1	4034.8	Unknown
2	0.13	5.0	785.6	Unknown
3	0.20	4.0	1204.2	Unknown
4	0.39	39.8	1086	Unknown
5	0.42	67.5	1552.1	Unknown
6	0.50	12.1	5038.0	Unknown
7	1.00	2.2	269.6	Unknown

Table: 10HPTLC –Flavonoids profile of the methanolic extracts of*Urgineaindica*(BX5)– bulb.

Peak	Rf	Height	Area	Assigned substance
1	0.12	187.9	36483.4	Unknown
2	0.20	142.9	17004.2	Unknown
3	0.33	30.7	13765.3	Unknown
4	0.39	55.7	2254.4	Unknown
5	0.44	54.7	1697.3	Unknown
6	0.50	11.5	1936.7	Unknown
7	0.58	4.7	2949.0	Unknown
8	0.70	12.8	1209.9	Unknown
9	0.79	12.8	647.7	Unknown
10	0.86	1.4	455.4	Catechin

Table :11Rf values of the standard Flavonoids:

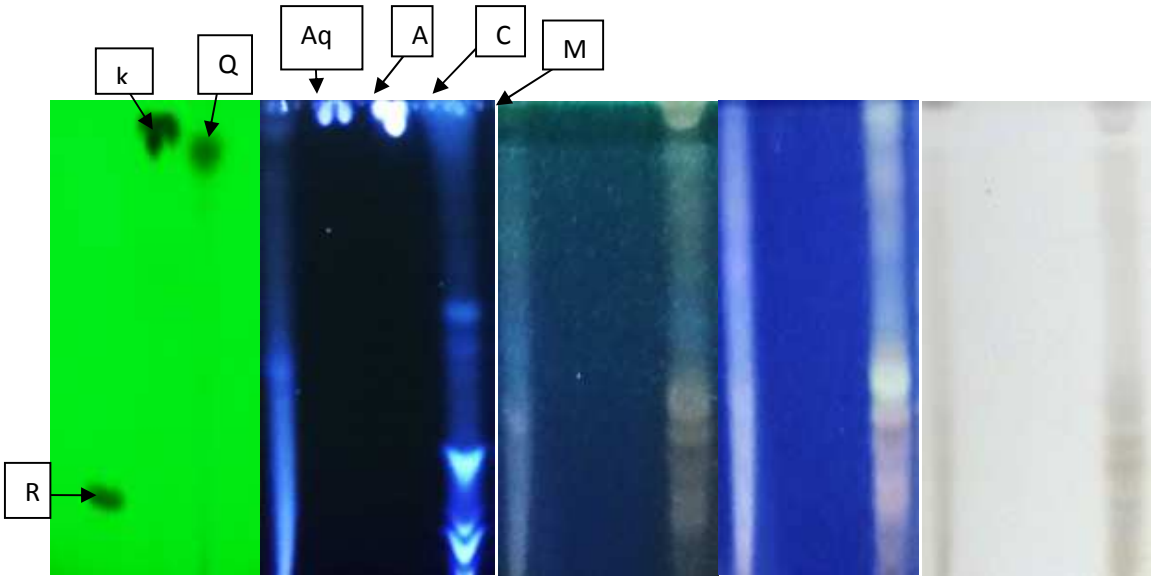
Peak	Rf	Height	Area	Assigned substance
1	0.96	2.2	32581	Quercetin
2	0.53	17.9	17245.2	Rutin
4	0.87	1.3	28144.1	Catechin
5	0.93	20.0	66563	Kaempferol
6	0.65	2.7	447.6	Hesperdin



A **B** **C** **D** **E**

Figure: 7 HPTLC studies on the flavonoids of the *Urgineaindica*.- bulb.(BX4)

A. HPTLC profile of the water,methanolic ,chloroform and acetone extracts *Urgineaindica*.- bulb Under UV254 BD.
B. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV366 BD.
C. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV254 AD.
D. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV366 AD.
E. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under light AD.



STD– R-Rutin , Q- Quercetin,K- Kaempferol, Aq-Aqueous extract, A-Acetone ,C-Chloroform, M-Methanol extract.

F G H I J

Figure: 8 HPTLC studies on the flavonoids of the *Urgineaindica*.- bulb(BX5).

F. HPTLC profile of the water,methanolic ,chloroform and acetone extracts *Urgineaindica*.- bulb Under UV254 BD.
G. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV366 BD.
H. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV254 AD.
I. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under UV366 AD.
J. HPTLC profile of the water,methanolic ,chloroform and acetone extracts of *Urgineaindica*.- bulb Under light AD.

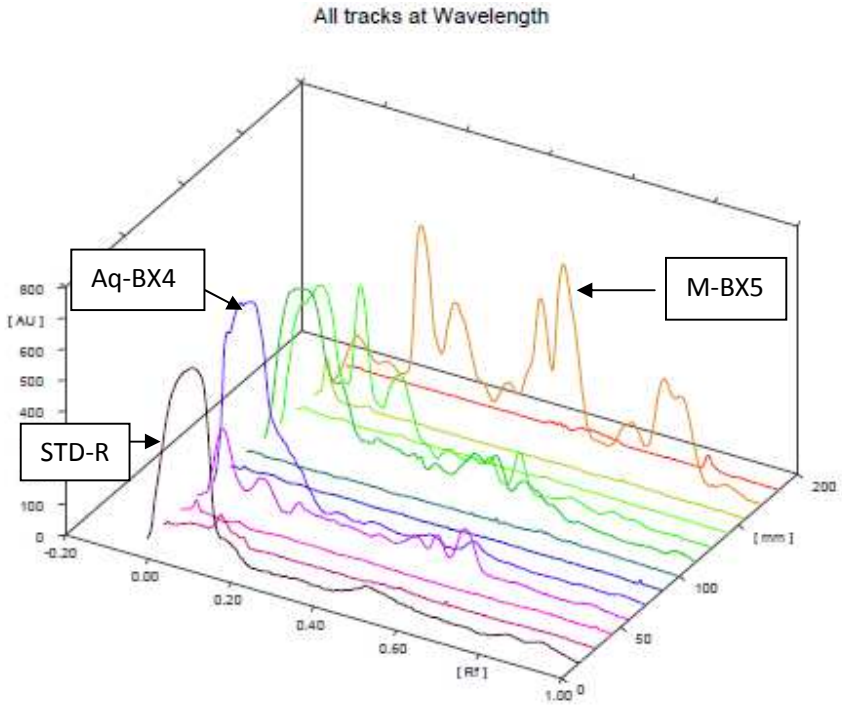


Fig.9: All tracks at wavelength 366 nm BD

Conclusion:

*Urginea*species have an ancient history of the multiple indigenous uses and is one of the most highly commercialized indigenous traditional medicines from India.Investigation of the phytochemicals and their biological activity has provided scientific support for many

of its traditional uses. The phytochemical analysis illustrates the occurrences of various micronutrients i.e. carbohydrates, vitaminC, vitamin E, flavonoids, phenols, glycosides, saponins and minerals i.e. Zn, Cu, Mn, Se, Fe. The significant antioxidant activity was observed due to adequate abundance of microelements and minerals in all extracts.Presence of selenium also reveals enhancing efficacyof antioxidant activity. The antioxidant activity in methanolic extract of BX5 and aqueousextract of BX4 showed significant results, which showed presence of strong potential of bioactive compounds. The presence of different flavonoids in bulbs of both species of *Urginea* showed its importance in therapeutic uses. The characterization of bioactive compounds and its importance in traditional therapy will be necessary in further study.

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