EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES

http://www.ejbps.com

ISSN 2349-8870
Volume: 2
Issue: 1
342-353
Year: 2015

HPLC ANALYSIS OF VITAMINS IN *URGINEA INDICA.*

M.N. Shiva Kameshwari*, Padma.S and R.Nijagunaiah

Department of Botany, Bangalore University, Bangalore.

Article Received on 12/12/2014 **Article Revised on 06/01/2015** **Article Accepted on 31/01/2015**

***Correspondence for Author**
M.N.Shiva Kameshwari
Department of Botany,
Bangalore University,
Bangalore.

ABSTRACT

The elements or chemicals present in medicinal plants play an important role in the treatment of diseases as described in Ayurveda. Some of the elements not only cure diseases but also add to the nutritional profile like vitamins, minerals and proteins. High Performance Liquid Chromatography (HPLC) has been applied for quantitative analysis of vitamins 3 fat soluble vitamins (A, E and K) and 7 water soluble vitamins (6 B Vitamins and Vitamin C) present in bulbs of *Urginea indica* a medicinal plant. HPLC method is a powerful technique in analysis of vitamins with high rate of accuracy. The data reveals the presence of various vitamins in varying concentrations and B_1, B_2, B_5, B_6, B_7 and B_{12} in low concentrations.

KEYWORDS: *Urginea indica,* HPLC, Vitamins.

INTRODUCTION

Urginea indica commonly called as Indian squill belongs to the family Hyacinthaceae and comprises of about hundred species. The genus is a geotype with a bulb and is endemic to certain floristic regions of the world distributed in remote and difficult terrains over the hills on the upper parts particularly on the slopes. In India it is found in southern and in peninsular part including the coastal belt as well as temperate regions in Himalayas.

The useful parts are bulbs which are excellent source of medicine with pharmaceutical and biocidal applications mainly as anticancer agent, expectorant, cardiac stimulant, hypertension, dyspepsia and arterio-sclerosis (Louria *et al*, 1985; Kendler, 1987 and Dorant *et. al*, 1996) in treatment of asthma (Marx et al., 2006), rheumatism, edema, dropsy, allergies

(Brodnitz *etal*, 1971), gout and to treat various other ailments (Benkeblia, 2004; Deepak and Shivakameshwari et al 2006).U*rginea being medicinally very important plant which has been by our work is gaining immense global importance in view of its multiple uses. The present study includes quantitative determination of vitamins particularly vitamin A, B complexes (B1, B2, B5, B6, B7 and B12), C and E from the dried powder of bulbs using the HPLC technique.

Vitamins are organic compounds required as vital nutrient in tiny amounts by many organism and forms integral part if diet (Lieberman and Bruning, 1990; Maton, et al, 1993). In humans there are thirteen vitamins classified as either water soluble (eight B vitamins and vitamin C) or fat soluble vitamins (Vitamin A, D, E and K). Each vitamin is typically used in multiple reactions and therefore has diverse biochemical functions. Some have hormone like or regulators of cell and tissue growth and differentiation (Vitamin A). Others function as antioxidants (Vitamin E and sometimes vitamin C). The largest number of vitamins (B-complex vitamins) functions as precursors for enzyme co-factors that help enzymes as part of prosthetic group (Devdas and Saroja, 1980; Oke, 1996; Bender and David, 2003 and Bolander, 2006).

The need of different vitamins varies depending on an individual's metabolism, age, sex,etc., WHO/FAO have made recommendations of daily allowances (RDA) for people aged 19-70 which is represented in Table:1 along with chemical name and deficiency diseases associated with different vitamins.

Table: 1

Sl.No.	Vitamin name	Chemical name	Deficiency diseases	Recommended Dietary Allowances (Age 19-70)
1	A	Retinoids (includes: retinol, retinal, retinoic acid, 3-dehydro retinol and its derivatives).	Night blindness, Keratomalacia.	900 µg
2	B1	Thiamine	Beri-beri.	1.2 mg
3	B2	Riboflavin	Ariboflavinosis.	1.3 mg
4	B3	Niacin	Pellagra.	16.0 mg
5	B5	Pantothenic acid	Paresthesia.	5.0 mg
6	B6	Pyridoxine	Anemia.	1.3-1.7 mg
7	B7	Biotin	n/a	30.0 µg
8	B9	Folic acid	Deficiency during pregnancy is associated with birth defects.	400 µg

9	B12	Cyanocobalamin	Megaloblastic anaemia.	2.4 µg
10	C	Ascorbic acid	Scurvy.	90.0 µg
11	D2-D4	Lumisterol, Ergocalciferol, Cholecalciferol, Dihydrotachy sterol, 7-Dihydro cholesterol.	Rickets.	5.0-10 µg
12	E	Tocopherol, Tocotrienol.	Deficiency is very rare, mild hemolytic anemia in new born infants.	15.0 mg
13	K	Naphthoquinone.	Bleeding diathesis.	120 µg

MATERIAL AND METHODS

The bulbs were collected from the Yediyur area of Bangalore, Karnataka, India and were grown in the Botanical garden of Department of Botany, Bangalore University, Bangalore, Karnataka, India under uniform environmental conditions. The fully grown bulbs of about 5 years old, weighing nearly 200gms and measuring 25cms in diameter were collected. The collected bulbs were shade dried for about 10 days and were powdered using mortar and pestle. This powder was used for further HPLC analysis of vitamins.

Quantification of vitamins A, E, B_1, B_2, B_5, B_6 ,B_7 and B_{12} was performed by HPLC method with SHIMADZU LC 20AD with dioade array detector (operated at 280nm) and injection valve with 20µl sample loop. Compounds were separated on 4.6mm×250mm, id, 5-µm pore size Zorbax SB RP-C18 column protected by a guard column containing the same packing. Quantification of VitaminC is performed using.

Vitamins are essential micronutrients. Vitamin A also called retinol, play an important role in bone growth, tooth development, reproduction, cell division, gene expression, regulating immune system. Skin, eyes and mucous membrane of the mouth, nose throat and lungs depend on vitamin A to remain moist Vitamin A is also important antioxidant that prevents cancer.

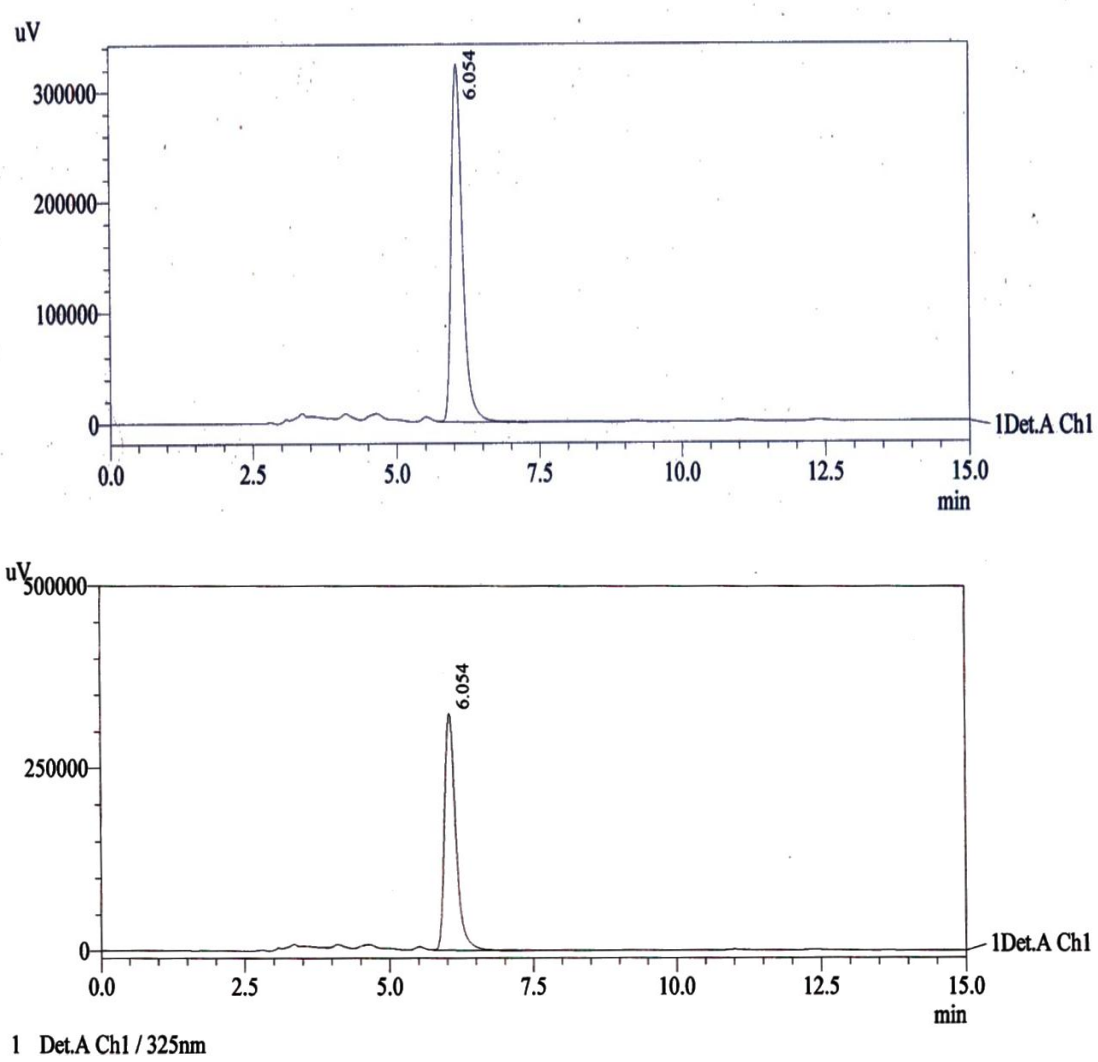
Vitamin A sources include carrots, pumpkin, winter squash, dark green leafy vegetables and apricots all of which are rich in beta- carotene.

Recent studies showed that vitamin A requirement may be increased due to hyperthyroidism, fever, infection, cold and exposure to excessive amounts of sunlight. People who are alcoholic and suffering from renal diseases should increase intake of vitaminA.Night blindness and rough skin may indicate a lack of Vitamin A.

RESULTS AND DISCUSSION

In the present study, quantitative estimation of vitamins was carried out by using HPLC technique. The sample was extracted from the 100 gms of bulbs of *Urginea indica* under different conditions and these extracts were analyzed using-----mg/ml sample concentration and compared with that of the standard using same concentration. The HPLC data are presented in Table: 2 and the Chromatograms obtained are shown in Figure: 1-8.

Sl.No	Vitamins	Quantity
1	A	5142 IU/Kg
2	B1	0.1 ppm
3	B2	0.547 ppm
4	B5	<0.01 ppm
5	B6	0.018 ppm
6	B7	1.0 ppm
7	B12	2.36 ppm
8	E	4341 IU/Kg



figurere:- 1 Vitamin A

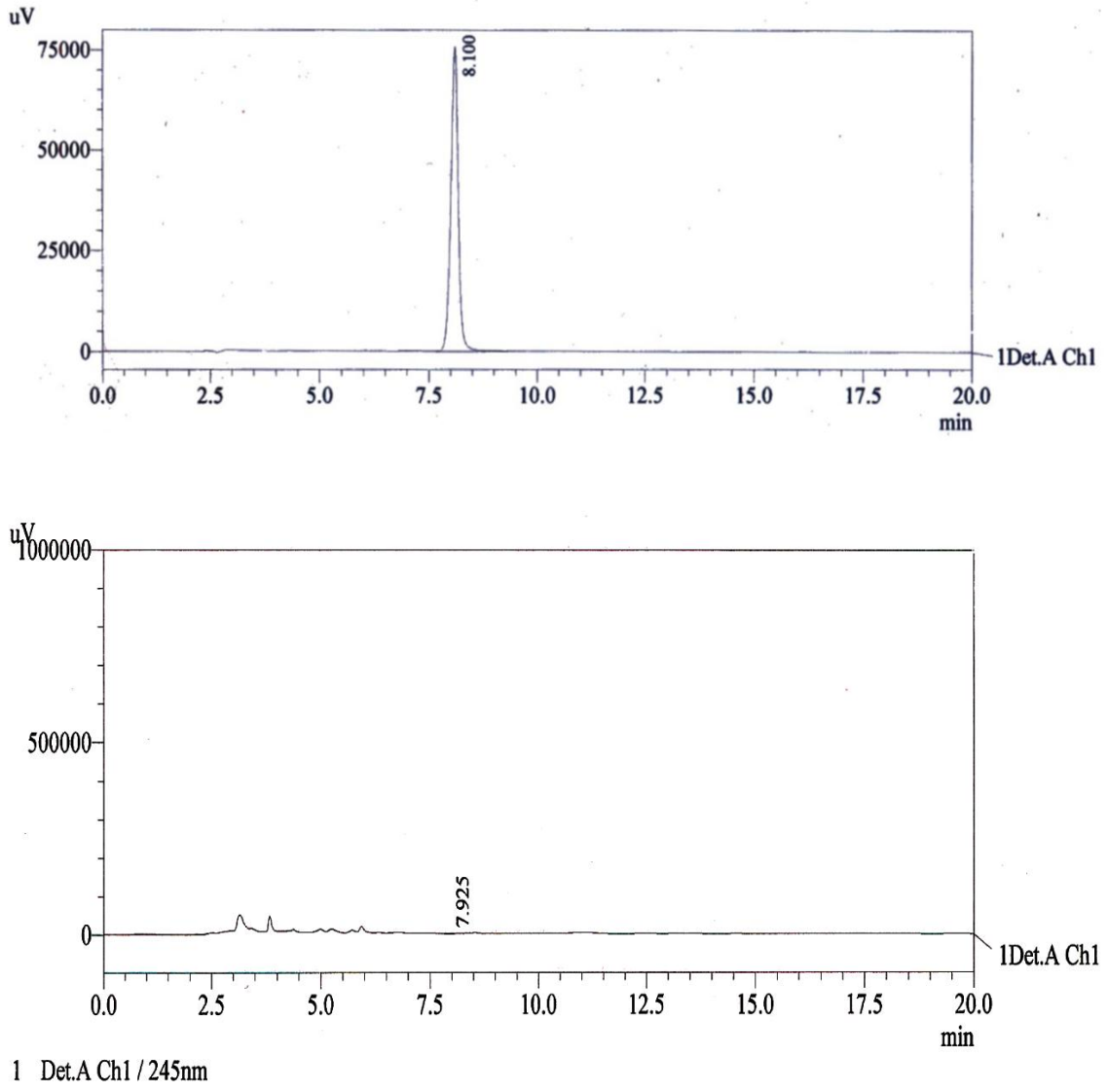
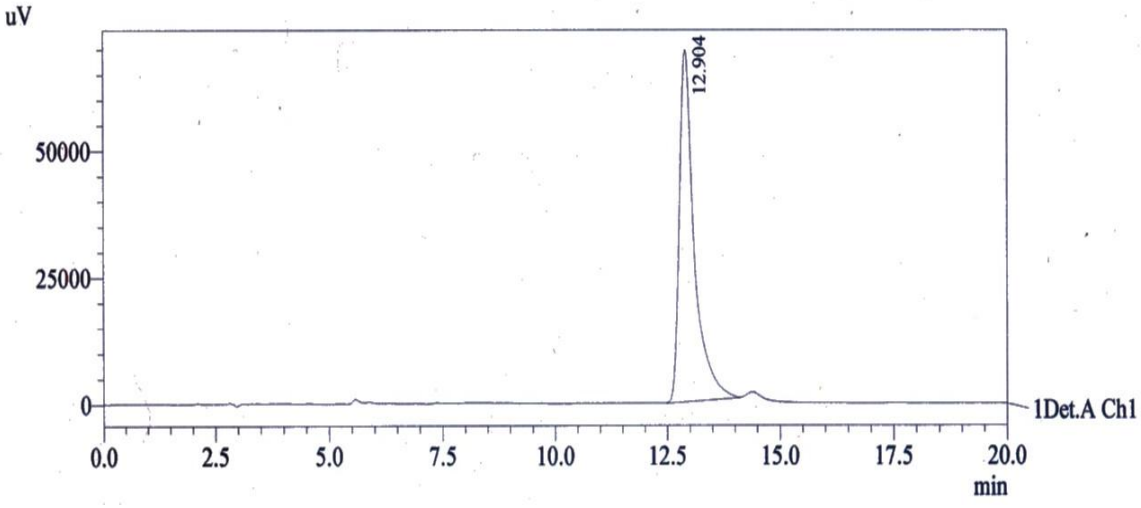


Figure:-2 Vitamin B1



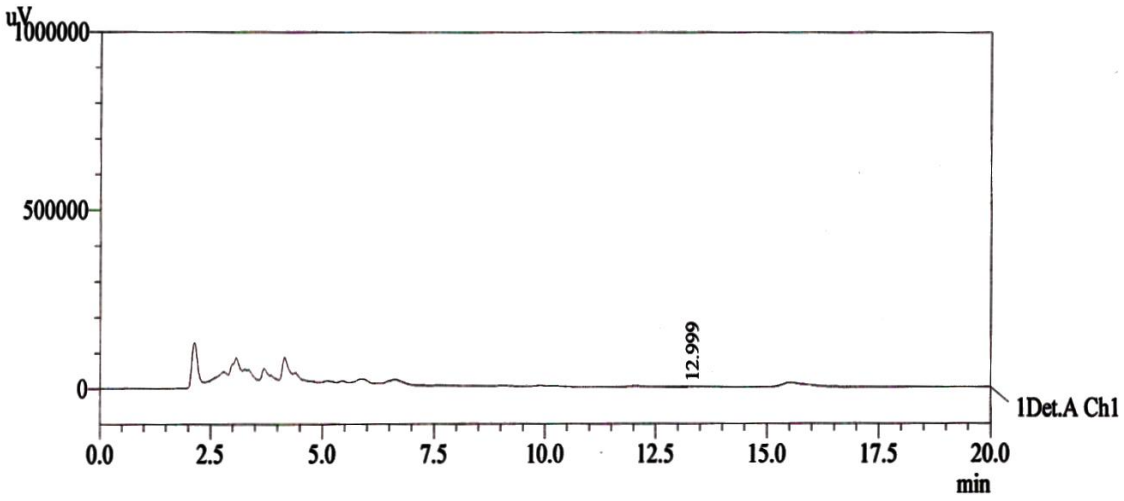


Figure:-3 Vitamin B2

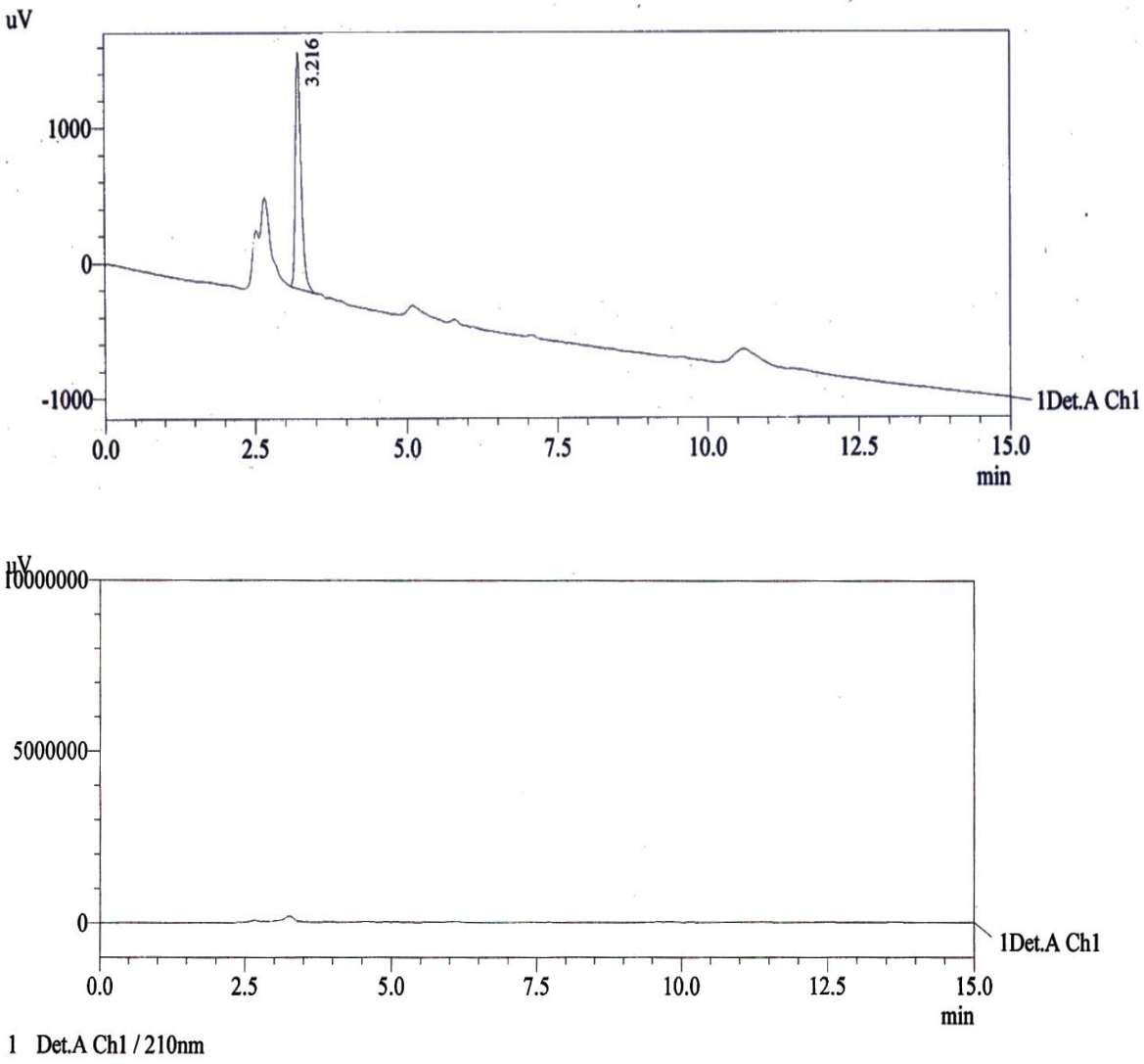


Figure:-4 Vitamin B5

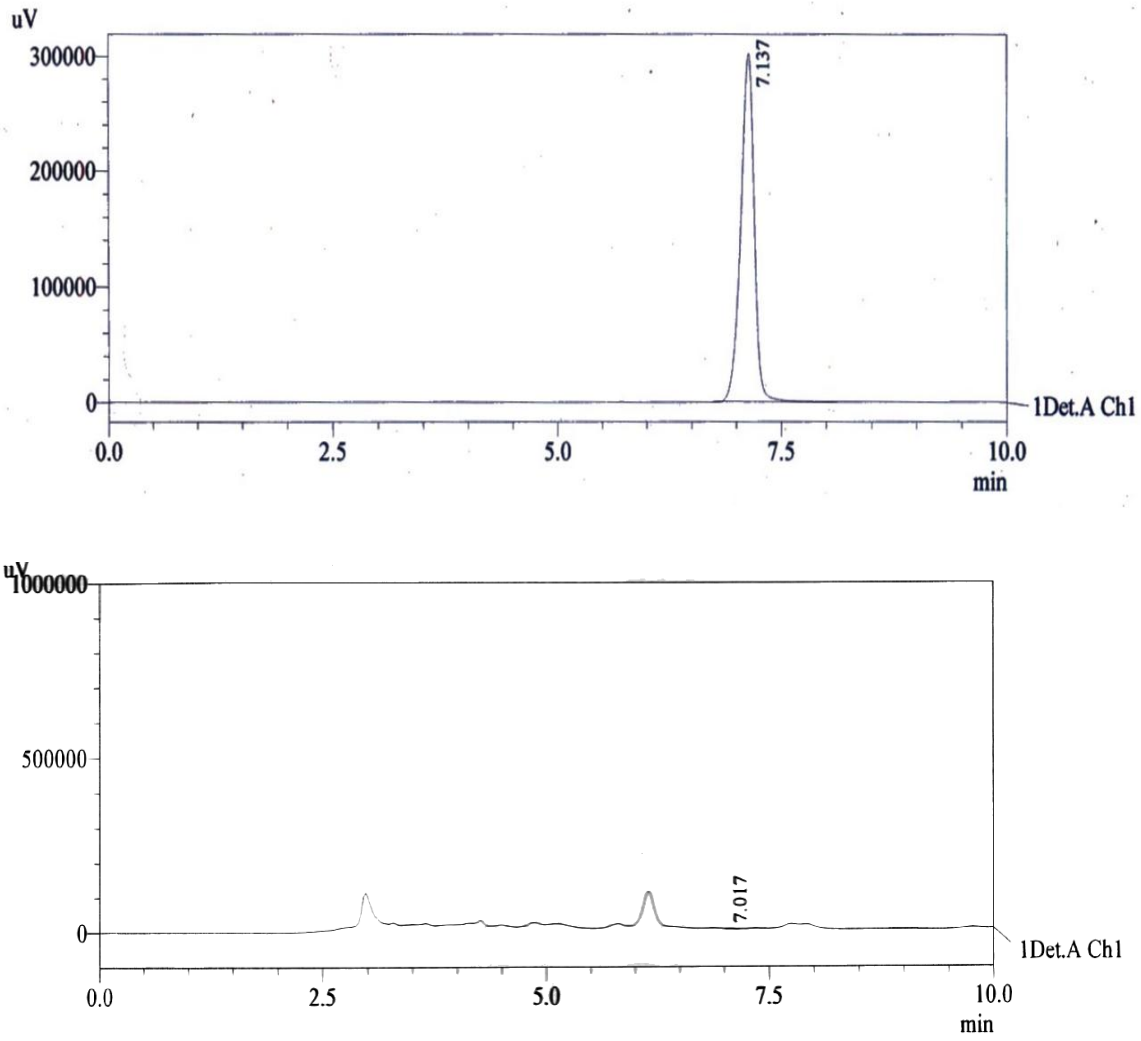
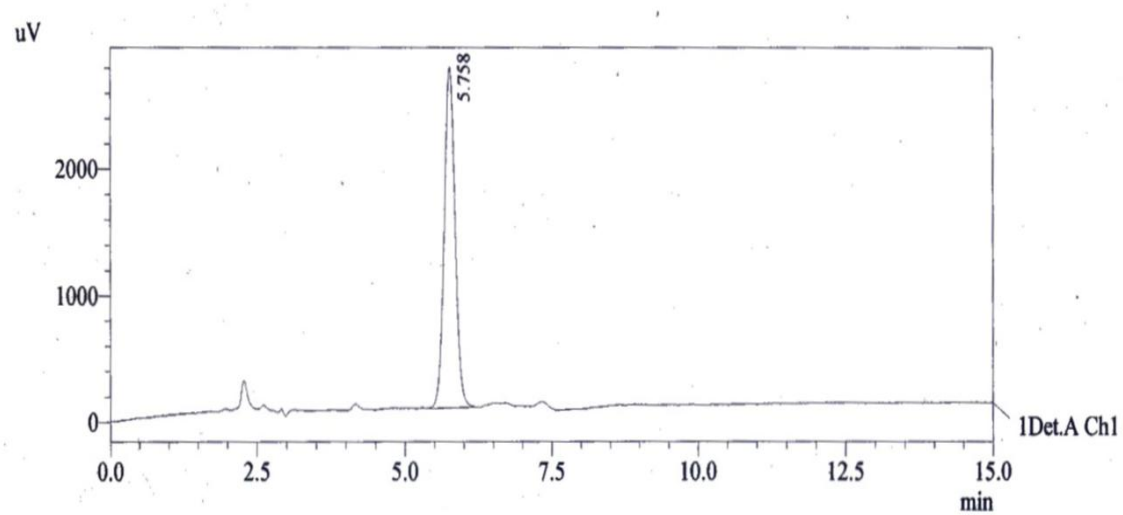


Figure:-5 Vitamin B6



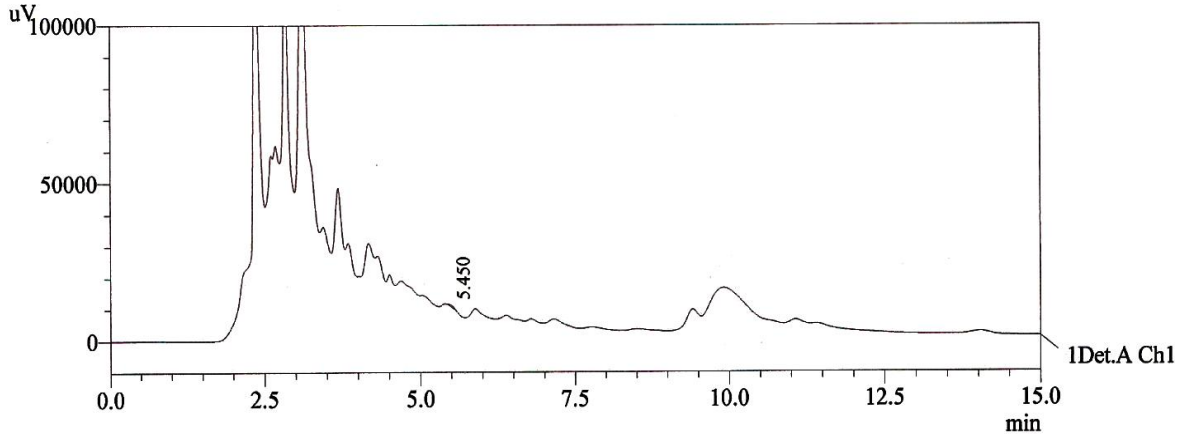
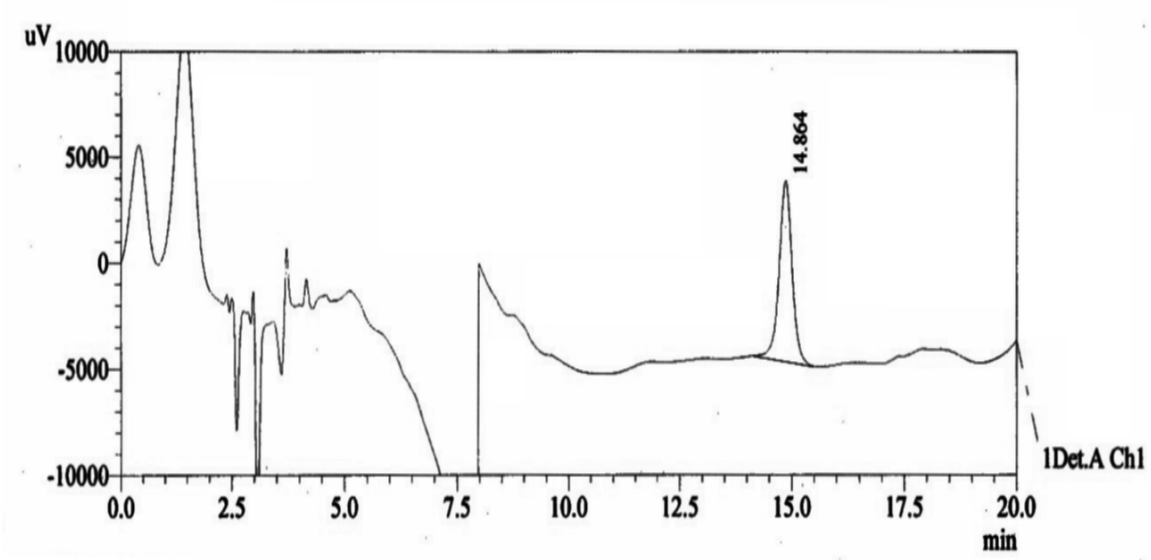
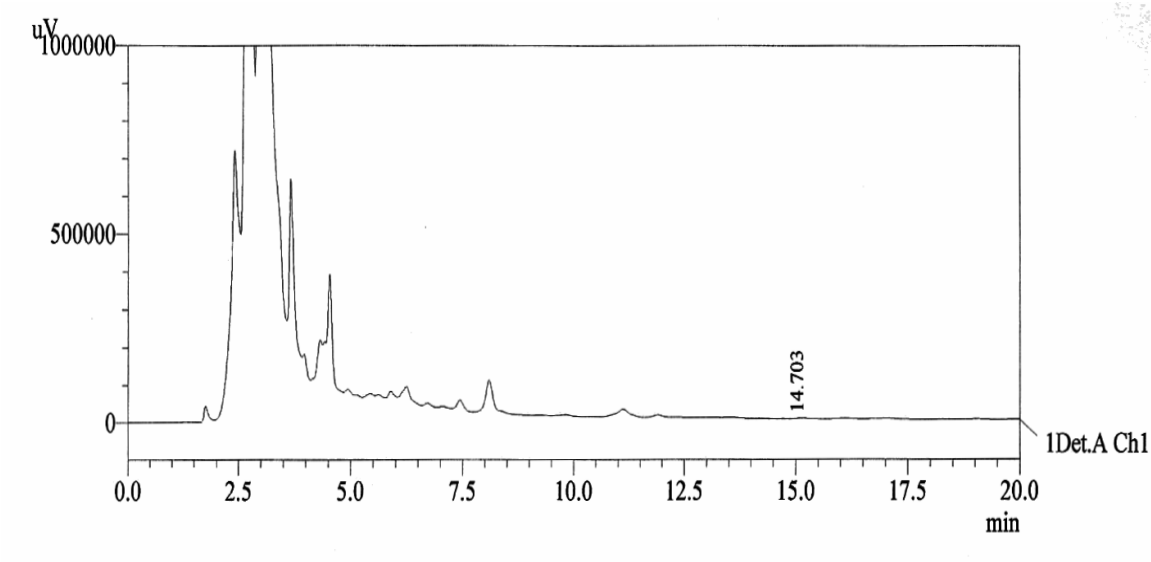


Figure:-6 Vitamin B12





Vitamin B7 (Biotin)

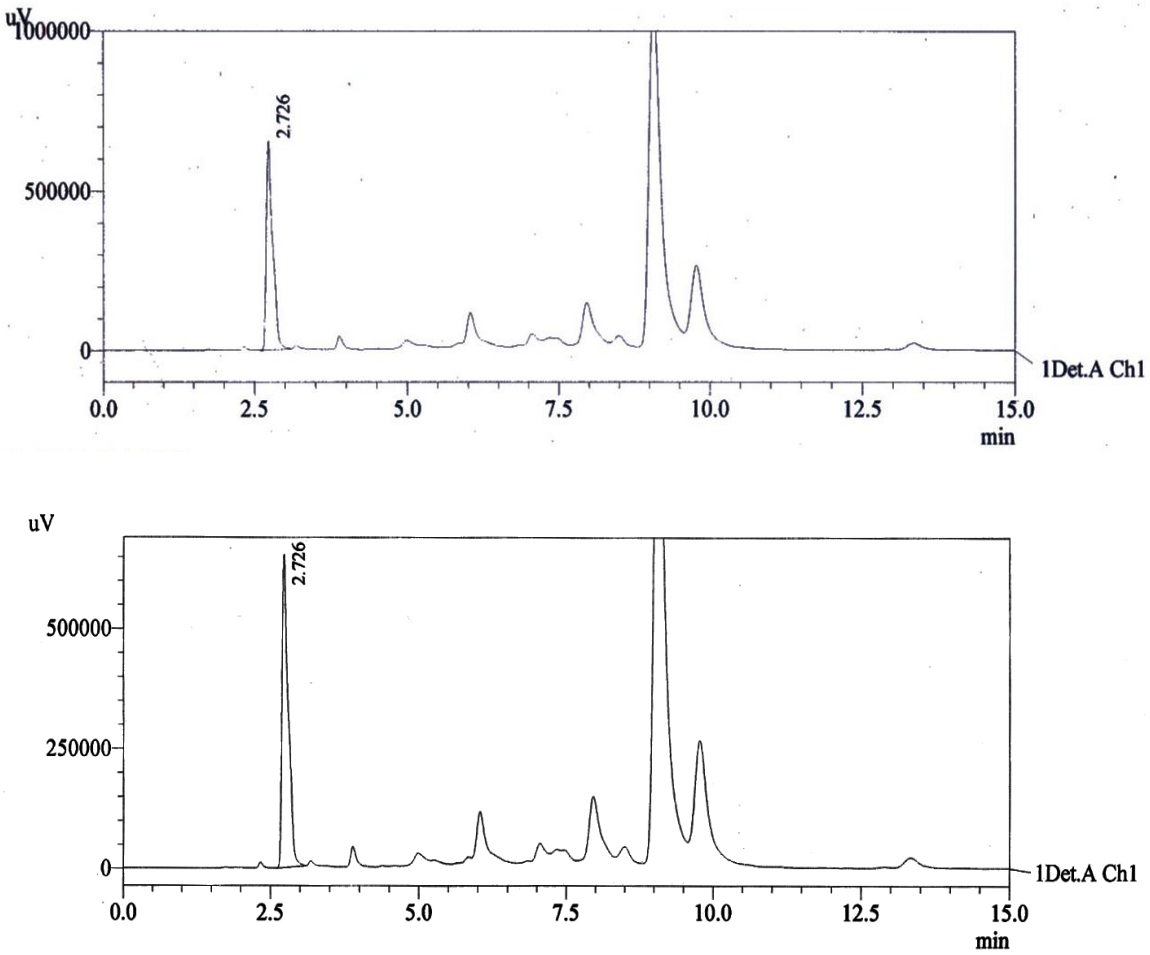


Figure:-8 Vitamin E

HPLC analysis of bulb extracts showed variation in the number of peaks as well as differences in the quantity of various vitamin contents which are as follows:

Vitamin A content recorded from the bulb extract was 5142 IU/Kg with peak retention time of 5.799 min.

Among the vitamin – B complexes/ series, vitamin B1, B2, B5, B6, B7 and B12 were detected among which B12 was found in increased quantity recording about 2.36 ppm with 5.450 min retention time, followed by vitamin B7 recording 1.0 ppm with 14.703 min retention time and vitamin B1 recording 0.1ppm with 7.925 min retention time.

Vitamin B2 and B6 content were comparatively lesser with B2 recording 0.547 ppm with peak retention time of 12.999 min and B6 recording 0.018 ppm with 7.017 min peak retention time. The least quantity of vitamin content was found in B5 which was less than 0.01 ppm with no proper peak retention time.

The quantity of vitamin E per 100 gm of bulb extract was found to be 4341 IU/Kg with 2.729 min of peak retention time. Vitamins C detected.

Many workers have worked on the analysis of various phytochemicals in *Urginea indica*. In 1972 Sathish and Bhakuni have identified compounds like alkaloids, lipids, octa cosanoic acid and β-sitosterol from the bulbs. Jha and Sen (1981) isolated three sterols such as campesterol, β-sitosterol and stigmasterol from this plant. Proscillardin A and scillaren A, scilliphaeoside and anhydro scilliphaeosidin were isolated and identified by Jha and Sen (1981 and 1983). Patil and Torne (1981) have performed phytochemical test and reported the presence of glycosides, mucilages and sugars in the bulbs. However, not much work on the vitamins has been carried out in *Urginea indica* except, in 1981 Patil and Torne has identified vitamins such as vitamin A, B1 and B2 from the bulbs of this plant. In this regard, the present work lights on the presence of other vitamins like A and E are fat soluble found in high concentration while B1, B2, B5, B6, B7, in low concentration which is water soluble. Among B vitamins B12 shows 236 ppm a slight increase in its concentration.

CONCLUSION

Over the years, reports have appeared in mainstream journals describing its medicinal properties. The present study suggests that, with acceptable/substantial amount of vitamin profile in the bulbs of *Urginea* and if it is consumed as vegetable in sufficient amount could contribute greatly towards meeting human vitamin requirement for normal body growth and provides adequate protection against diseases arising from vitamin deficiency. However, this is not possible as its utility as a food product is not accepted and recommended due to the presence of chemical scilliroside which is said to have toxic effect on consumption. With such limiting factors with compounds capable of eliciting deleterious effects in man and animals, present report can only be an additional data which mentions about the vitamin profile of the plant. This report can also inspire other researchers to work with regard to remove or curb the toxic effects of this species in order to make it consumption friendly vegetable. If this is possible it can help to adequately establish their importance in human nutrition and provide basis for their utilization as non conventional vegetable.

ACKNOWLEDGEMENT

Dr.Shiva kameshwari is thankful for the financial support under UGC grants and to the Post Graduate Department of Botany, Bangalore University, Bangalore for providing the necessary facilities.

REFERENCES

1. Bander and David, A. 2003. Nutritional biochemistry of the vitamins. Cambridge, U.K. Cambridge University Press.
2. Benkeblia, N. Antimicrobial activity of essential oil extracts of various Onions (*Allium cepa)* and Garlic (*Allium sativum*). *lebensm- WISS- U- Technol,* 2004; 373.
3. Bolander, F.F. "Vitamins: Not just for enzymes". *Current opinion investigating drugs,* 2006; 7(10): 912-5.
4. Brodnitz, M.H. and Pascale, J.V. Thiopropanal Soxide: A lachrymatory [sic] factor in onions. *J Agric Food Chem*; 1971; 19: 269–72.
5. Deepak, A.V. and Salimath, B.P. *Biochimie,* 2006; 88(3-4): 297-307.
6. Devdas, R.P. and Saroja, S. Availability of Fe and â carotene from *Amaranthus t*o children. In Emmaus P (Ed), Proceeding of the 2nd *Amaranthus* Conference, Rodales Press Inc., 1980; 15-21.
7. Dorant .E, Van Der Brandt P.A., Goldbohm R.A., Sturmans F. Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterolog.* 1996; 110: 12–20.
8. Jha, S. and Sen, S. Bufadienolides in different chromosomal races of Indian squill. *Phytochemistry*, 1981; 20: 524-526.
9. Jha, S. and Sen, S. Sterols in different cytological races of *Urginea indica*. *Phytochemistry,* 1981; 20: 1442-1443.
10. Jha, S. and Sen, S. Quantitation of principal bufadienolides in different cytotypes of *Urginea indica. Planta Medica*, 1983; 47(1): 43-45.
11. Kendler B.S. Garlic *(Allium sativum)* and onion *(Allium cepa):* A review of their relationship to cardiovascular disease. *Prev Med,* 1987; 16: 670–85.
12. Lieberman, S. and Bruning, N. 1990. The real vitamin and mineral book. New York. Avery group. 3.
13. Louria, D.B., Mc Anally, J.F., Lasser, N. et al., Onion extract in treatment of hypertension and hyperlipidemia: A preliminary communication. *Curr Ther Res,* 1985; 37: 127–131.
14. Marx, J., Pretorius, E. and Bester, M.J. *J. Ethnopharmacol.*, 2006; 104(3): 315-21.
15. Maton, Anthea, Maryanna Quon Warner, David La Hart, Jill D. Wright. 1993. Human Biology and Health. Eagle wood cliffs. New jersey, USA Hall.
16. Oke, O.L. Chemical studies on the more commonly used vegetables in Nigeria. *Afr.Sci.Ass,* 1966; 11: 42-48.
17. Patil, M.M. and Torne, S.G. Vitamin contents in *U. indica* cytotypes. *Indian drugs,* 1981; 18: 345-350.

18. Patil,M.M., Torne, S.G., Chemical analysis of *Urginea indica* (roxb.) Kunth. Cytotypes. *Curr. Sci.,* 1981; 50: 956-958.
19. Sadashivam and Manicham, A. Vitamins-Acorbic acid, colorimetric analysis, Biochemical methods. New age International Publishers, 1997; 184-186.
20. Satish, S. and Bhakuni, D.S., Constituents of Indian and other plants. *Phytochemistry*, 1972; 11(9): 2888-2890.