ISSN: 2249-5746
International Journal of Ayurvedic and Herbal Medicine 7:3 (2017) 2590–2596
Journal homepage: http://www.interscience.org.uk
UGC Approved Journal
Impact factor: 4.415

Identification and Determination of Bioactive Polyphenols of *V. Vinefera* for Phyto-therapeutic Applications

[1] Handore A.V. and [2]* Khandelwal S. R.

[1, 2] Department of Microbiology, HPT Arts and RYK Science College, Nashik -422005, MS, India.

Abstract

Vitis vinifera is one of the important economic fruit crop having widespread application in variety of health diseases and disorders. Since an ancient times, *Vitis vinefera* is significantly used in *Ayurveda* due presence of bioactive polyphenols. .Although, its fruits and seeds has been used in various *Ayurvedic Rasayan* drugs and formulations, scanty research has been carried out on presence of polyphenols in aerial parts of this plants and their study as a remedial agent. The present study deals with identification and determination of bioactive polyphenols in Black and White (Green) cultivar of *V.vinefera* .Organic extracts were prepared by soaking the dry powder into 90% Methanol. Qualitative analysis of different parts of *V. vinefera* viz. leaf lamina, stem and petiole was carried out by phytochemical screening. In the quantitative analysis, Folin-Ciocalteau method was used to determine total phenolic content whereas, total flavonoid content was estimated by aluminum chloride colorimetric assay. Free radical scavenging activity of extract was carried out by DPPH assay and accumulation of bioactive compounds in different regions was studied by anatomical analysis. Results of phytochemical screening of both cultivars showed presence of bioactive polyphenols viz. flavonoids, ellagic acid, tannin along with other phenolic compounds in high abundance. In the white cultivar total phenolic content of petiole was found to be highest (1.44± 0.19 mg/g GAE) whereas, leaves showed lowest content (0.38± 0.11 mg/g GAE).In the black cultivar, leaves showed highest content of total phenols (1.57± 0.05 mg/g GAE) and stem showed lowest content i.e. 0.41± 0.07 mg/g GAE. Total flavonoid content of petiole of white cultivar was observed to be highest (0.15 ±0.05mg/g quercetin equivalent) as compared to leaves and stem. Whereas, leaves of black cultivar showed almost high content of flavonoid i.e. 0.26 ±0.09 mg/g quercetin equivalent. Order of radical scavenging activity for both cultivar was, petiole (79.24%)>stem (75.10%)>leaf lamina (72.37%) and stem (83.03%)> petiole (80.40 %)> leaf lamina (62.42%) respectively .Amount of flavonoids (quercetin) in petiole of white cultivar was found to be 3.27 ppm by HPLC analysis. Findings of the current study revealed that aerial parts of *V. vinefera* are rich source of various bioactive polyphenolic compounds with promising therapeutic potential. Therefore irrespective to economic boundaries, these aerial parts can be efficiently used in for phyto-therapeutic applications.

Keywords: *Vitis vinefera*, Aerial parts, Bioactive polyphenols, Antioxidant, Phyto-therapeutic

Introduction

Polyphenols are found ubiquitously in plants their regular consumption has been associated with a reduced risk of a number of chronic diseases, including cancer, cardiovascular disease and neurodegenerative disorders. Rather than exerting direct antioxidant effects, the mechanisms by which polyphenols express these beneficial properties appear to involve their interaction with cellular signaling pathways and related machinery that mediate cell function under both normal and pathological conditions [1]

Vitis vinifera is one of the important fruit crop linked with human history during the evolutionary development of humankind. According to the scientific evidences, fruit berry, seeds and skin of *V, vinefera* are used in the different *Ayurvedik Rasayan* drugs and formulations since an ancient times. This plant contains various bioactive polyphenolic compounds which are highly accumulated in different parts such

as berry, skin, seeds, leaves, stems containing polyphenolic compounds viz. flavonoids predominantly, (+)-catechins, (−)- epicatechin and procyanidin polymers along with the stilbene compounds like resveratrol and some phenolic acids. These grape polyphenols possess numerous biological activities and health-promoting potential. It caused arrest of G1-phase cell cycle, decreased the cell cycle regulators such as cyclins D1/D2/E and C (dks, hyper-phosphorylated pRb proteins, AP-1,and MEK1>ERK1/2signaling)and enhanced the p21WAF1/CIP1. Moreover, increased production of 8-oxo-7, 8-dihydro-2′- deoxy guanosine in UVA-irradiated genomic DNA of HaCat human keratinocyte cells is also reported [2-3] .They also employs antioxidant mechanism through (i) scavenging peroxyl radicals and lipid alkoxyl (ii) chelating metal ions (iii) producing α-tocopherol during reduction of the α- tocopheroxyl radical. [4]

On basis of clinical trials, this plant is considered as important source of various bioactive phytochemicals (viz .flavonoids, tannins, resveratrol, and some phenolic acids including ellagic acid) [5].These polyphenolic phytochemicals are known for their anit-oxidative , antimicrobial, anti- inflammatory capacity [6] anti-carcinogenic [7]and antihypertensive activities. [8] .In addition to this, ,these polyphenolic compounds exhibit an effect on cell signaling pathways and on the gene expression [9-10].In 1990, Marusic, a well-known herbal medicine practitioner stated that the prime material for preparation of herbal remedies should be grapes, flowers, leaves and vine tendrils.[11]

Unfortunately, scanty research has been carried out on presence of polyphenols in aerial parts of this plants and their study as a remedial agent. So, the present work has been focused on qualitative and quantitative analysis of various bioactive polyphenols as well as their free radical scavenging activity (antioxidant activity) for studying the therapeutic potential of *V.vinefera.*
e

Materials and Methods

Collection of Plant Material

Fresh and healthy aerial plant parts (leaf lamina, stem and petiole) of White and Black cultivars of *V. vinefera* were randomly collected from vineyards of Nashik valley, Maharashtra India during June 2016.

Preparation of Plant Extract

Plant parts were cleaned, cut into small pieces and shade dried at room temperature for fifteen days and grounded to fine powder to store in air tight bags. Organic extracts are prepared by soaking the dry powder (10%) into 90% Methanol and incubated at room temperature with gentle shaking for 72 h. The supernatants obtained was used for further analysis.

Phytochemical analysis

Extracts were tested for the presence of various bioactive polyphenols by using reported standard methods like, flavonoids by Shinoda Test [12] tannins by Gelatin Test [13] ellagic acid [14] and presence of other phenolic compounds were detected by Ferric Chloride Test [15].These tests were identified by visual observation of colour change or by precipitate formation on addition of specific reagents to the test solution.

Determination of total phenolics

Total phenolics content was determined by Folin-Ciocalteau method of Zheng and Wang. Absorbance of the test sample was measured at 725 nm and content of phenolics in extracts was calculated using a Gallic acid (0.1-1.0 mg mL^{-1}) standard curve ,and the results were expressed in terms of gallic acid equivalent (mg of GAE/g of extract).[16]

Quantitative analysis of flavonoids.

The total flavonoid content was estimated using aluminum chloride assay [17].Absorbance of the test sample was measured at 510 nm and total flavonoid content was calculated as quercetin equivalents from a calibration curve of quercetin. The calibration curve was prepared in the same manner using 0.1-1 mg mL^{-1} of quercetin solutions in methanol.

In Vitro Antioxidant Assay

The antioxidant potential of plant extracts (leaf -lamina, stem and petiole) were estimated by the free radical scavenging ability or donation of hydrogen ions with reference to the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity by the spectrophotometric method reported by Santanu Sannigrahi.et al., with few modifications .[18]Briefly, plant extract (100µl) was added into 100 µL methanolic solution of DPPH (0.1mM) final volume was made up to 2 mL with methanol. Whole mixture was shaken vigorously and incubated (30 min) in dark at room temperature. Absorbance was measured at 517 nm and percentage of DPPH scavenging activity or % inhibition was calculated by following equation,

Percentage DPPH scavenging activity=(A_0–A_1)/ A_0 x100
(Where, A_0 =Absorbance of the control,A 1=Absorbance of the sample)

HPLC Analysis

HPLC Analysis was performed on Analytical Technologies Ltd.-HPLC 3000 series gradient binary systems. Column configurations were Grace,RP-18,250mm× 4.6 mm (ID),Particle size 5 µm held at room temperature using the solvent system, Methanol:Water (90:10)(v/v).Flavonoid-quercetin (50 ppm) was used as reference. Sample was detected at 270 nm with 20ul of sample injection. at 0.8 ml/min(Isocratically).

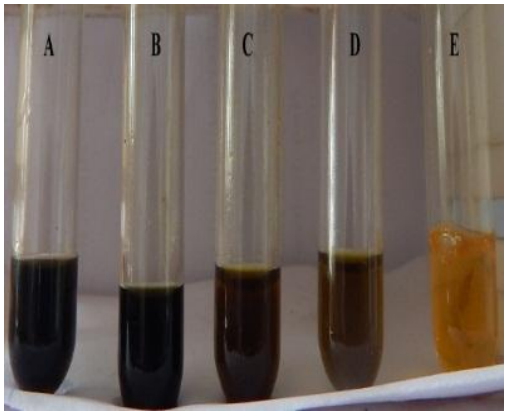
Anatomical analysis

For anatomical analysis of fresh samples (Mature leaf lamina, stem and petiole), very thin transverse sections were obtained with sharp razor followed by staining with safranin. Microscopic observation was carried out to study the accumulation of bioactive compounds in different regions.

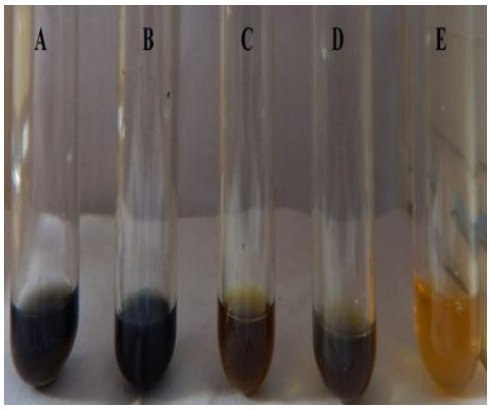
Results and Discussion

Phytochemical analysis

Presence of bioactive polyphenols was detected by visual observation of color change or by precipitate formation on addition of the specific reagents to the test solution. It was found that all the aerial parts of both cultivars showed presence of Flavonoids, Ellagic acid, Tannin and other phenolic compounds in high abundance. **(Fig.1.)**



[White Cultivar]



[Black Cultivar]

A .Positive Control , B .Leaf lamina ,C. Stem, D. Petiole , E. Negative Control

Phytochemical Detection Tests for Bioactive Polyphenolic compounds

Determination of total Phenolics

Total phenolic content was determined by Folin-Ciocalteau method and expressed as mg gallic acid equivalent(GAE) per gram of extract using a standard curve of gallic acid ($y = 0.5724x + 0.3721$,$r^2 = 0.968$). Results obtained showed that total phenolic content in the petiole of white cultivar was highest (1.44± 0.19 mg/g GAE) whereas, leaves showed lowest content (0.38± 0.11 mg/g GAE).In black cultivar, leaves showed highest content of total phenols (1.57± 0.05 mg/g GAE) and stem showed lowest content i.e. 0.41± 0.07 mg/g GAE. (**Table.1.)**

Sample	Total Flavonoid Content mg/g quercetin equivalent	Total Phenolic Content mg/g Gallic acid equivalent
White Cultivar		
A (Leaf Lamina)	0.09 ±0.02	0.38± 0.11
B (Stem)	0.13 ±0.03	0.87± 0.01
C (Petiole)	0.15 ±0.05	1.44± 0.19
Red Cultivar		
I (Leaf Lamina)	0.26 ±0.09	1.57± 0.05
II (Stem)	0.22 ±0.05	0.41± 0.07
III (Petiole)	0.26 ±0.06	1.07± 0.19

**All values were expressed as the mean of triplicates ± standard deviation (SD).*

Table.1.Total Phenolic Content and Total Flavonoid content of Red and White Cultivar of *V.vinefera*

Quantitative analysis of flavonoids.

The total Flavonoid content was determined by aluminum chloride colorimetric method and expressed as quercetin equivalents in mg/g dry powder, and fractions using a standard curve of quercetin ($y = 0.3369x + 0.0149$,$r^2 = 0.9701$). Total flavonoid content of petiole of white cultivar was observed to be highest (0.15 ±0.05mg/g quercetin equivalent) as compared to leaves and stem. Wheras, leaves of black cultivar showed almost high content of flavonoid i.e. 0.26 ±0.09 mg/g quercetin equivalent. (**Table.1)**

Antioxidant Activity of Extract:

The DPPH radical scavenging activity of the polyphenolic extract of different aerial parts of *V.vinefera* is carried out in triplicates. Ascorbic acid was used as reference compound. It was observed that petiole of white cultivar, showed highest radical scavenging activity or % inhibition (79.24%) in comparison with stem (75.10%) and leaf lamina (72.37%).Whereas, in red cultivar, highest radical scavenging activity was shown by stem (83.03%) as compared to petiole and leaf which was 80.40 % and 62.42 % respectively. The observed reduction of DPPH by the extract was either due to the transfer of hydrogen atom or transfer of an electron as phenolic compounds are effective hydrogen donors, which makes them good antioxidants .Thus, dietary polyphenolic compounds could facilitate the endogenous antioxidant system by stimulating transcription.

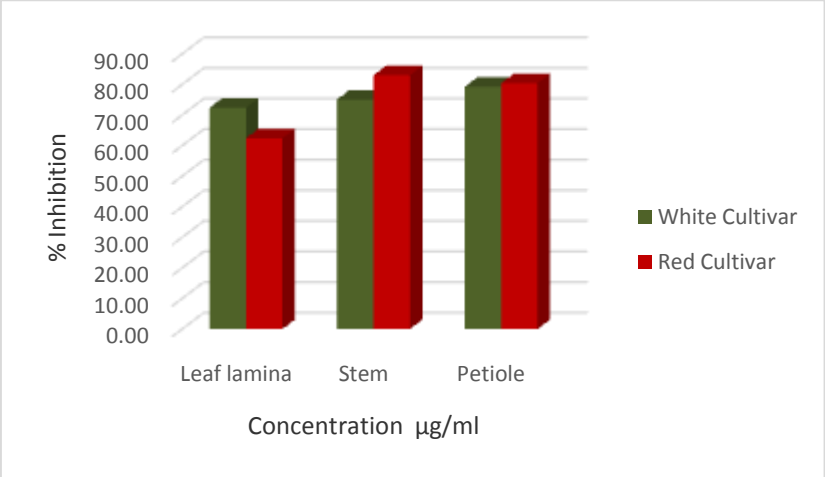
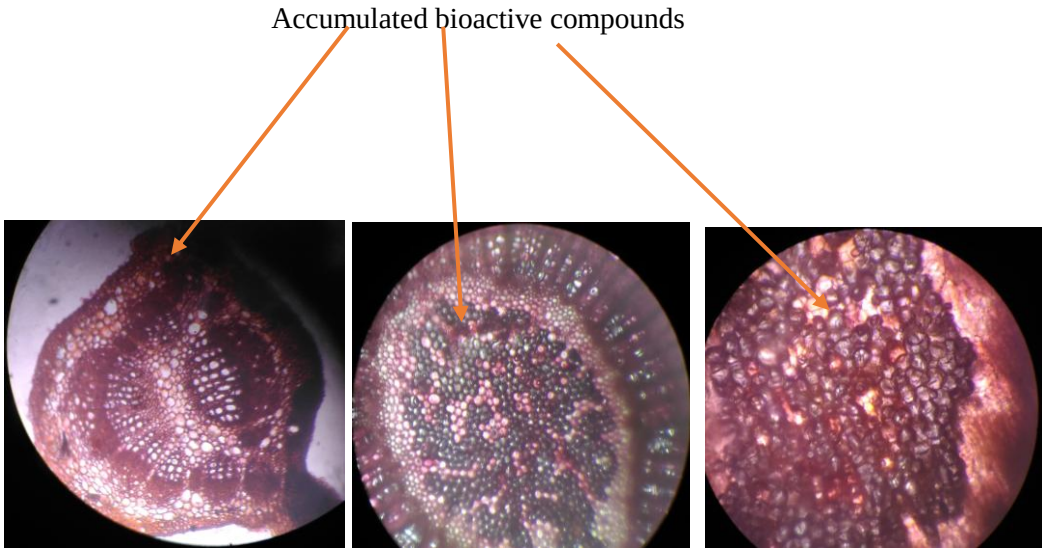


Fig.2. Antioxidant potential of Aerial parts of *V.vinefera*

Anatomy of aerial plant parts

The anatomical analysis of aerial parts showed variations in accumulation of bioactive compounds in leaf lamina, stem and petiole. Transverse section of the leaf lamina and stem showed comparatively less accumulation of bioactive compounds in different regions whereas transverse section of petiole showed highest accumulation of bioactive compounds in epidermis, hypodermal cortex, parenchymatus cortex, phloem, pith region etc.

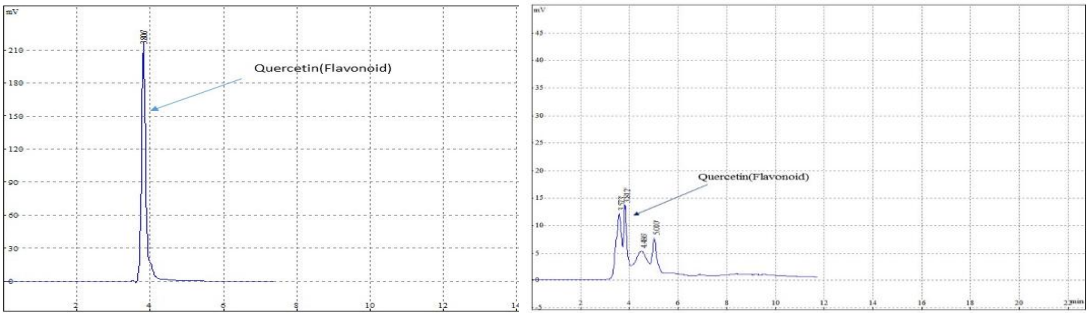


A. Leaf lamina B. Stem C. Petiole

Fig.3. Transverse section of *V. vinefera* (Black Cultivar)under 40 X magnification:

HPLC Analysis

Detection and quantification of flavonoid (quercetin) content of petiole of white cultivar was carried out by use of HPLC. The chromatogram was obtained at 270nm and identified by comparing the retention time of sample with standard Rt =3.8min. Flavonoid (Quercetin) concentration was found to be 3.27 ppm.



(a) (b)

Fig.4. (a) HPLC chromatogram of Flavonoid standard(Quercetin) at 270nm , Rt =3.8min

(b)HPLC chromatogram of petiole extract(white cultivar), at 270nm , Rt =3.8min

Conclusion

The present study was carried out for qualitative as well as quantitative analysis of various bioactive compounds in the aerial parts of *V.vinefera* .Phytochemical screening of almost all the aerial parts of both cultivars showed presence of flavonoids, Ellagic acid, Tannin and other phenolic compounds in very high abundance. Anatomical analysis of the leaf lamina and stem showed comparatively less accumulation of bioactive compounds whereas, transverse section of petiole showed highest accumulation of bioactive compounds. Total flavonoid content of petiole of white cultivar was observed to be highest (0.15 ±0.05mg/g quercetin equivalent) as compared to leaf lamina and stem. Whereas, leaves of black cultivar showed almost high content of flavonoid which was 0.26 ±0.09 mg/g quercetin equivalent .It was found that total phenolic content in the petiole of white cultivar was highest (1.44± 0.19 mg/g GAE) whereas, leaves showed lowest content (0.38± 0.11 mg/g GAE).In the black cultivar, leaf lamina showed highest content of total phenols (1.57± 0.05 mg/g GAE) and stem showed lowest content i.e. 0.41± 0.07 mg/g GAE. Findings of the quantitative study demonstrated that although, fruit berry and seeds of *V.vinefera* are reported as rich sources of medicinally essential compounds ,aerial parts of this plants have significant amount of bioactive polyphenols which are clinically proved as therapeutic agents due to their anti-oxidative, anti-microbial, anti-inflammatory, anti-carcinogenic and anti-hypertensive activities. Therefore irrespective to economic boundaries, aerial parts of *V.vinefera* can be efficiently used for phyto-therapeutic applications.

Acknowledgment

The authors are grateful to Prin. V. N. Suryavanshi and Dr. L.P.Sharma, HOD, Department of Microbiology, H.P.T .Arts and R.Y.K. Science College, Nashik, India for providing the necessary facilities. We acknowledge Ms. V.S. Jagtap and J.W.Baviskar for the assistance. Authors are highly thankful to Mr. D.V.Handore, Research Mentor, Sigma Winery Pvt. Ltd. Nashik for valuable scientific inputs.

References

1. David V., Ana R., Giulia C, Maria Jose O. and Jeremy P. E., Polyphenols and Human Health: Prevention of Disease and Mechanisms of Action, *Nutrients* 2010, (2):1106-1131
2. Ahmad, N., Adhami V.M., Afaq F., Feyes D.K., and Mukhtar H.. Resveratrol causes WAF-1/p21-mediated G1-phase arrest of cell cycle and induction of apoptosis in human epidermoid carcinoma A431. Cells. 2001.(7): 1466–1473.
3. Adhami, V.M., Afaq F., and Ahmad N. Involvement of the retinoblastoma (pRb)-E2F/DP pathway during anti proliferative effects of resveratrol in human epidermoid carcinoma (A431) cells. Biochem. Biophys. Res. Commun,2001.(288):579–585.
4. Bors, W., Heller W., Michel C., and Saran M.. Flavonoids as antioxidants: determination of radical scavenging efficiencies. In: Methods in Enzymology, vol. 186. Academic Press, New York,

1990:343–355.
5. Doshi, P., Adsule P., and Banerjee K.. Phenolic composition and antioxidant activity in grapevine parts and berries (*Vitis vinifera* L.) cv Kishmish Chornyi (*Sharad Seedless)* during maturation. Int. J. Food Sci. Technol,2006.(41): 1–9.
6. Xia, EQ., Deng, GF., Guo, YJ., Li, HB, Biological Activities of Polyphenols from Grapes, Int J Mol Sci, 11 ,2010:622–646
7. Agarwal, C, Veluri, R., Kaur, M., Chou, SC., Thompson, JA., Agarwal, R., Fractionation of high molecular weight tannins in grape seed extract and identification of procyanidin B2 – 3, 3'-di-O-gallalte as a major active constituent causing growth inhibition and apoptotic death of DUI 45 human prostate carcinoma cells, Carcinogenesis, 2007,(28):1478 – 1484.
8. Peng, N., Clark, JT., Prasain, J., Kim, H., White, CR., Wyss, JM., Antihypertensive and cognitive effects of grape polyphenols in estrogen-depleted, female, spontaneously hypertensive rats, Am J Physiol Regul Integr Comp Physiol, 2005, 289(3):771-775
9. Soobrattee, MA., Neergheena, VS., Luximon-Rammaa, A., Aruomab, OI., Bahoruna, T, Phenolics as potential antioxidant therapeutic agents: Mechanism and actions, Mutation Research Fundamental and Molecular Mutagen, 2005.(579) :200–213.
10. Dell A., M., Galli, GV., Vrhovsek, U., Mattivi, F., Bosisio, E, In vitro inhibition of human cGMP-specific phosphodiesterase-5 by polyphenols from red grapes, J of Agricultural and Food Chemistry ,2005,(53) :1960–1965
11. Marušic, R. Ljekovitim B, Through herbal medicine to health. Milac, M.(ed.), Zagreb: Mladost,1990:358
12. Sabiha S., Aftab M. Ahmad, M. Asif, Mohd A.and Ibne S., Physicochemical and phytochemical standardization of berries of Myrtus communis Linn, J Pharm Bioallied Sci. 2012,.4(4) :322–326
13. Bhandary S. K.., Kumari S. N. , Bhat V. S. , K.P Sharmila. , Bekal M. P., Preliminary Phytochemical Screening Of Various Extracts Of Punica Granatum Peel,Whole Fruit And Seeds ,Nujhs 2012, 2, (4):34-38
14. Rimjhim S., Kumari N. and. Jainendra K, Preliminary Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum, IOSR Journal of Applied Chemistry, 2014,(7):110-13
15. Usman H, Abdulrahman F I, and Usman A, Qualitative Phytochemical Screening and In Vitro Antimicrobial Effects of Methanol Stem Bark Extract of Ficus Thonningii (Moraceae), Afr. J. Trad. CAM. 2009,6 (3): 289 - 295
16. Zheng, W., Wang, Y.S, Antioxidant Activity and Phenolic Compounds in Selected Herbs. J. Agric. Food Chem. 2001,(49) :5165-5170
17. Zhishen J, Mengcheng T and Jianming W, The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chemistry. 1999, (64):555-559
18. Sannigrahia Sa., Mazuder U..K., Palc D. K., Paridaa S.and Jaina S., Antioxidant Potential of Crude Extract and Different Fractions of Enhydra fluctuans Lour, Iranian Journal of Pharmaceutical Research 2010, 9 (1): 75-82