Available online at www.scholarsresearchlibrary.com

Scholars Research Library

European Journal of Zoological Research, 2012, 1 (4):105-110
(http://scholarsresearchlibrary.com/archive.html)

Scholars Research Library
ISSN: 2278–7356

Analysis of vitamins by hplc and phenolic compounds, flavonoids by HPTLC in *Microcosmus Exasperatus*

V.K. Meenakshi*, S. Gomathy, S. Senthamarai, M. Paripooranaselvi and K.P. Chamundeswari

Department of Zoology, A.P.C.Mahalaxmi College for Women, Tuticorin, Tamilnadu, India

---

ABSTRACT

Microcosmus exasperatus, a simple ascidian was analysed for the presence of water soluble vitamin B; fat soluble vitamins A, D, E and K by High Performance Liquid Chromatography and phenolic compounds, flavonoids by High Performance Thin Layer chromatography. Fat soluble vitamins D_3, K and water soluble vitamin riboflavin and thiamine were noticed in high concentration. HPTLC studies revealed the presence of phenolic compounds such as Gallic acid, Ferulic acid and Caffeic acid. Rutin, Isoquercitrin and Quercetin were some of the flavonoids identified.

Keywords: *Microcosmus exasperatus*, HPLC, HPTLC, vitamins, flavonoids

---

INTRODUCTION

Ascidians or Sea squirts are marine sessile filter feeding animals. The edible part of ascidians contains a variety of amino acids, minerals and fatty acids which have considerable benefits to human body. Some ascidians are widely enjoyed as food in Japan, particularly in Hokkaido and Tohoku districts because of the high amount of protein, carbohydrate and other essential micronutrients [1]. Like other marine food products, ascidians are delicious, relatively easily digestible and offer minerals, iodine and vitamins [2]. *Microcosmus* belongs to the family Pyuridae and it includes eight Mediterranean species [3,4]. Three species, namely *M. polymorphus*, *M. sabatieri* and *M. vulgaris* are edible and commercially harvested in the Mediterranean since the 1st century AD [5]. *M. polymorphus* are eaten in large quantities in France and Italy and consumed raw [6]. The sea pineapple (*Halocynthia roretzi*) is an edible ascidian consumed primarily in Korea. Most of the ascidians are utilized as such as food in various countries. A review of literature shows that studies on the nutritional value [7], biochemical components [8], GC- MS analysis [9] and antidiabetic activity [10] of *Microcosmus exasperatus* are available. But investigation of its edible value based on the presence of vitamins has not been studied. In the present study, an attempt has been made to evaluate the presence of Vitamins in *Microcosmus exasperatus* using HPLC and phenolic compounds, flavonoids by HPTLC.

MATERIALS AND METHODS

Collection of material: Samples of *Microcosmus exasperatus* were collected from the Tuticorin harbour. Identification up to the species level was carried out based on the key to identification of ascidians [11]. A voucher specimen AS 2240 has been deposited in the museum, Department of Zoology, A.P.C. Mahalaxmi College for Women, Tuticorin - 628002. The animals were cleaned with sea water, shade dried and homogenized to get a coarse powder which was stored in an airtight container and used for further investigations.

HPLC Technique: Shimadzu CLASS-VP V6. 13 SP2 instrument was used to carry out HPLC analysis employing the following conditions: An analytical Column C18 with a mobile phase Solvent A (20%) 1 gm of pentane sulfonic acid, 1 gm of Hexane sulfonic acid in 1000 ml distilled water and Solvent B (80%) 1gm of pentane sulfonic acid

and 0.5 gm of Hexane sulfonic acid in 1000 ml Methanol; Flow rate-1.0 ml/min; UV detector-variable wave length detector 285 nm, Column compartment temperature 40^0C; Injection volume – 10 micro liter for water soluble vitamins. Column 4.6 x 75 mm Zorbax Eclipse XDB-C18,3.5 im; Mobile phase A=water, B=methanol, Gradient at 0 min 90% B, at 20 min 100% B, Column wash at 21 min 90% B; Flow rate-1.0 ml/min; UV detector-variable wave length detector 210 nm, standard cell, Column compartment temperature 20^0C; Stop time 21 min, Post time 5 min; Injection volume – 5 micro liter for fat soluble vitamins.

Water soluble Vitamin - B - Standard Preparation: Standards of water soluble vitamins - Niacin, Pyridoxine, Thiamine, and Riboflavin were purchased from Sigma Company. 10 mg of the standard was dissolved in 10 ml of methanol. From this, 100 µl was taken and made up to 10 ml with methanol. This was injected into the HPLC.

Sample Preparation: 500 mg of dry powder of *Microcosmus exasperatus* was taken and 10 ml of mobile phase A (1 gm of pentane sulfonic acid, 1 gm of Hexane sulfonic acid made up to 1000 ml with distilled water) was added and kept in sonicator for 30 min at 40°c and made up to 100 ml. 1gm of pentane sulfonic acid and 0.5 gm of Hexane sulfonic acid made up to 1000 ml with Methanol and used as Mobile phase B.

Fat Soluble Vitamin - A, D_3, E and K - Standard Preparation: Standards of Fat soluble vitamins - A, D, E and K were procured from Sigma Company. 10 mg of the standard was dissolved in 1 ml of methanol.

Sample Preparation: One gram of dry powder of *Microcosmus exasperatus* was taken. 5 ml of Hexane was added and mixed thoroughly for 10 minutes. The upper hexane layer was transferred to small test tubes and evaporated to dryness. The residue obtained was dissolved in 50 µl of 95% methanol (Mobile Phase: A-Water; B- Methanol).

HPTLC Technique: Using a Camag microlitre syringe and linomat IV applicator the samples were spotted in the form of 7 mm width bands on a precoated silica gel plate 60F 254 [10 cm X 10 cm with 0.2 mm thickness, E.Merck]. The plates were developed in a solvent system in CAMAG glass twin trough chamber previously saturated with the solvent for 30 minutes. The distance travelled by solvent system was fixed as 8 cm. TLC plates were air dried and scanning was performed on a Camag TLC Scanner at absorbance of 280 nm.

Standard Preparation: 10 mg of the **s**tandards of flavonoids and phenolic compounds were weighed and transferred to a 10 ml volumetric flask, dissolved in methanol and the volume made up to 10 ml to get 1 mg/ml solution.

Sample Preparation: One gram of the dry powder of *Microcosmus exasperatus* was extracted with methanol by soxhlet extraction. The extract was filtered through Whatman filter paper No.42. The volume of the filtrate was made up to 10 ml in a volumetric flask and used for HPTLC analysis.

RESULTS AND DISCUSSION

HPLC chromatogram of the water soluble vitamins in *Microcosmus exasperatus* (Figure 1.) showed four peaks corresponding to the four vitamins identified. Their retention time, peak area, height and concentration are given in Table 1. The B complex vitamins detected were niacin, pyridoxine, thiamine and riboflavin. Among these, the concentration of riboflavin was 31.79 µg/gm and thiamine 3.10 µg/gm whereas niacin and pyridoxine were found in low concentration 0.62 and 0.02 µg/gm respectively. Table 2 and Figure 2 shows the results of HPLC analysis of fat soluble vitamins. The presence of three peaks corresponds to the vitamins A, D_3 and K identified. Of these, vitamin D_3 (75 µg/gm) occurred in higher concentration followed by Vitamin A (20 µg/gm) and K (15.7 µg/gm). Vitamin E was found below detectable level.

Vitamins are essential nutrients which plays an important role in the day to day life of every human. Vitamin B_1/thiamine is important as it helps the body cells convert carbohydrates into energy. It is also essential for the functioning of the heart, muscles, and nervous system [13]. Not getting enough thiamine can leave one fatigued and weak. Vitamin B_2 or riboflavin is important for body growth, reproduction and red cell production. It also helps in releasing energy from carbohydrates. In the present study the concentration of riboflavin was found to be comparatively very high (31.79 µg/gm) indicating that value added food prepared out of *Microcosmus exasperatus* could be used as a supplement in those suffering from growth retardation, reproductive abnormalities or decreased RBC synthesis. Vitamin A prevents night blindness and is essential for the normal functioning of the body epithelia [12]. Vitamin K is essential for blood clotting and Vitamin D plays a vital role by acting on target organ like bones, kidneys, intestinal mucosa, strengthens the bones and prevents osteomalacia [13], protects against Alzheimer's and Parkinson's diseases. High content of Vitamin D_3 (75 µg/gm) observed in *Microcosmus exasperatus* is suggestive of its importance as a nutritive diet in the absorption of calcium in the intestine and formation of normal bones and

teeth in man. Analysis of vitamins by HPLC revealed the presence of water soluble vitamins - niacin, pyridoxine, thiamine and riboflavin and fat soluble vitamins A, D_3 and K of which riboflavin and D_3 were present in higher concentration.

HPTLC chromatogram of the phenols and flavonoids in *Microcosmus exasperatus* is presented in Figure 3. The Rf values, Peak area and concentration of the different phenols and flavonoids identified is given in Table 3. In HPTLC studies, six peaks were noticed in the chromatogram. Three peaks represent phenols such as gallic acid, ferulic acid and caffeic acid wheras the remaining three peaks have been identified as the flavonoids rutin, isoquercitrin and quercetin. Among the three phenols the maximum peak area (6014.29) was observed for gallic acid followed by ferulic acid (696.51), caffeic acid (622.81) with the concentration of 21.77 µg/g, 0.97 µg/g and 0.34 µg/g respectively. Of the three flavonoids maximum peak area (33287.00) corresponding to quercetin, rutin (163.83), isoquercitrin (922.21) was observed with the concentration of 265.95 µg/g, 1.22 µg/g and 0.378 µg/g.

Ferulic acid is a natural phenol suggested to have direct antitumor activity against breast and liver cancer in animal and invitro studies [14,15]. It also effectively scavenges deleterious radicals and suppresses radiation induced oxidative reaction serving as an important antioxidant [16]. Gallic acid is a type of phenolic acid, commonly used in the pharmaceutical industry [17]. It possesses significant antioxidant activity and may protect the liver from the harmful effects of free radicals that are formed as a result of various metabolic processes in the body [18]. Ointments for psoriasis and external haemorrhoids contain gallic acid. Caffeic acid is an antioxidant showing anti-ischemia reperfusion, anti-thrombosis, antihypertension, anti-fibrosis, antivirus and antitumor properties [19]

Rutin and quercetin exhibit anti-inflammatory, antihepatotoxic, antiulcer, antiallergic, antioxidant, antidiabetic, antiviral, reduce low density lipoprotein and provides protection against cardiovascular mortality [20,21,22,23,24]. Isoquercitrin has demonstrated dose-dependent protective effect against oxidative endothelial injury [25]. It has also been shown to protect venular endothelium from inflammatory products released by activated blood platelets and polymorphonuclear granulocytes [26].

Both Quercetin and Isoquercitrin glucosidase inhibitors [27].Isoquercitrin plays an important protective role against lipid peroxidation and oxidative stress [28]. Isoquercitrin regulates blood glucose and lipids levels and also improves the function of pancreatic islets. Isoquercitrin may be useful in treating type 2 diabetes mellitus [29].

FIGURE 1: HPLC CHROMATOGRAM OF WATER SOLUBLE VITAMINS IN *MICROCOSMUS EXASPERATUS*

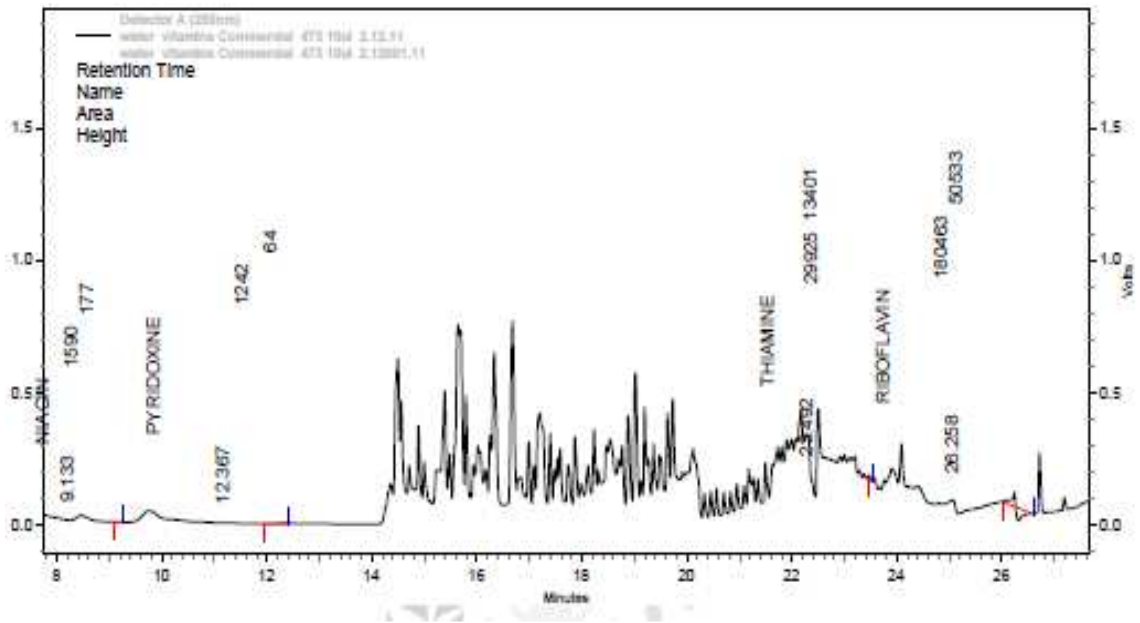


TABLE 1: WATER SOLUBLE VITAMINS IN *MICROCOSMUS EXASPERATUS*

Pk#	Retention Time	Area	Height	Name	Concentration (ug/gm)
1.	9.133	1590	177	Niacin	0.62
2.	12.367	1242	64	Pyridoxine	0.02
3.	23.492	29925	13401	Thiamine	3.10
4.	26.258	180463	50533	Riboflavin	31.79

TABLE 2: FAT SOLUBLE VITAMINS IN *MICROCOSMUS EXASPERATUS*

Pk#	Retention Time	Area	Height	Name	Concentration (ug/gm)
1.	2.908	5031	2814	Vitamin A	20.0
2.	13.775	13791	49	Vitamin D3	75.0
3.	20.417	1258	1	Vitamin E	LOD
4.	22.742	161071	14220	Vitamin K	15.7

FIGURE 2: HPLC CHROMATOGRAM OF FAT SOLUBLE VITAMINS IN *MICROCOSMUS EXASPERATUS*

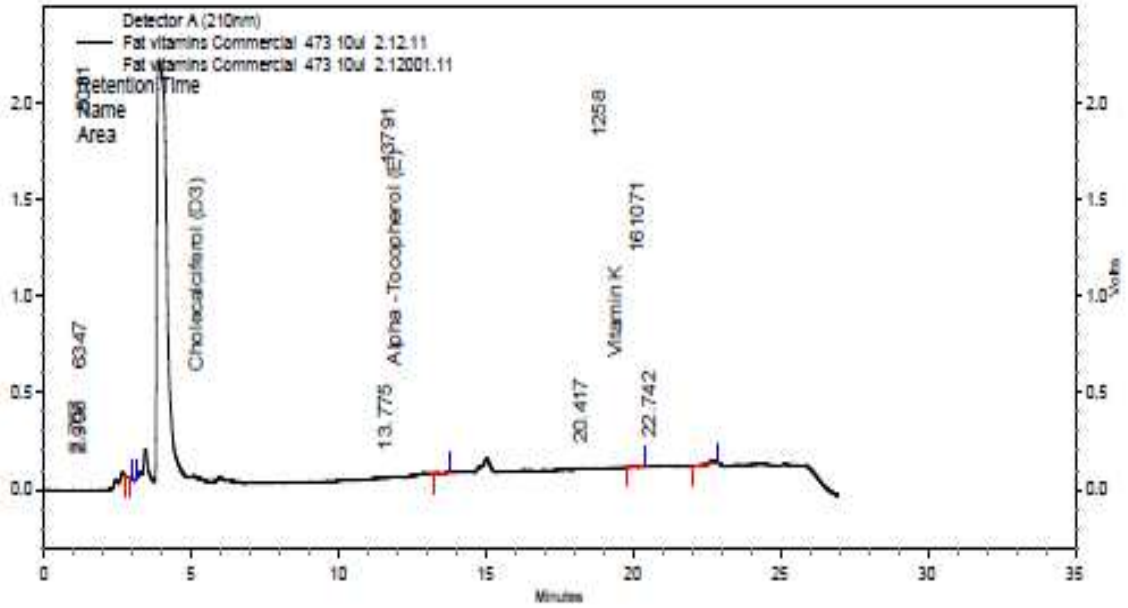
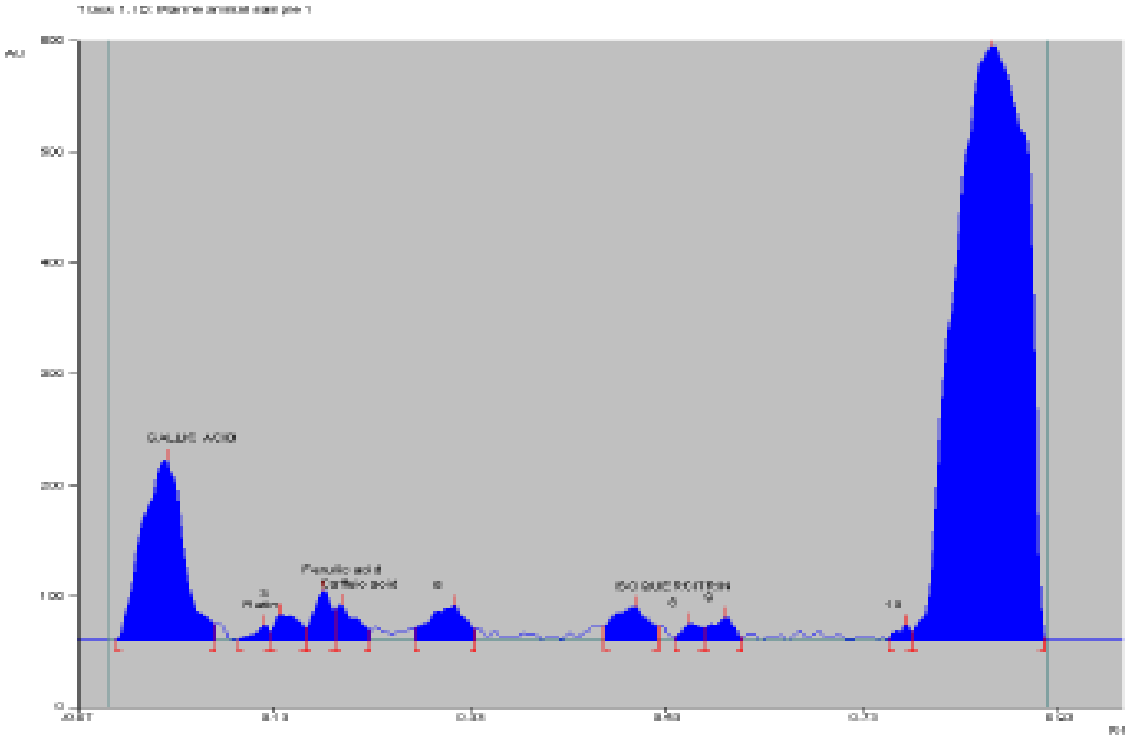


TABLE 3: PHENOLIC COMPOUNDS AND FLAVONOIDS IN *MICROCOSMUS EXASPERATUS*

Chemical Constituents	Rf	Peak area	Concentration µg/g
Gallic acid	0.02	6014.29	21.77
Ferulic acid	0.18	696.51	0.97
Caffeic acid	0.21	622.81	0.34
Quercetin	0.87	33287.00	265.95
Rutin	0.12	163.83	1.22
Isoquercitrin	0.50	922.21	0.378

FIGURE 3: HPTLC CHROMATOGRAM OF PHENOLIC COMPOUNDS AND FLAVONOIDS IN *MICROCOSMUS EXASPERATUS*



CONCLUSION

An analysis of the content of vitamins, phenolic compounds and flavonoids in *Microcosmus exasperatus* in the present study suggests that this simple ascidian which is commonly available throughout the year from Tuticorin coast could serve as an important source of beneficial nutrients.

Acknowledgement
The authors express their deep sense of gratitude to the University Grants Commission, New Delhi F.No. 39-588/2010 (SR) for financial assistance and Dr. S. Kumaravel, Senior Scientist, Indian Institute of Crop Processing Technology, Thanjavur, India for providing facilities for HPLC and HPTLC studies.

REFERENCES

[1] K Nanri; J Ogawa; Nishikawa T. *Nanki Seibutu*, **1992,** *34*, 135.
[2] VK Meenakshi. *IJBT*, **2010,** *1*, 29-33.
[3] A Koukouras; E Voultsıadou-Koukoura; T Kevrekidis; Vafidis, D. *Annals of Institute of Oceanography*, **1995,** *71(1)*, 19-34.
[4] A Izquierdo-Muñoz; M Díaz-Valdés; Ramos-Esplá RR. *Aquatic Invasions*, **2009,** *4(1)*, 59-64.
[5] D Vafidis; C Antoniadou; Chintiroglou C. *Journal of the Marine Biological Association of the United Kingdom*, **2008,** *88(5)*, 1043-1051.
[6] Herdem Aslan Cihangir; A Andres Izquierdo Muñoz, Maria; A Pancucci Papadopoulou; Alfonso Ramos Esplá, Elif Can Yılmaz. *Turkish Journal of Fisheries and Aquatic Sciences*, **2011,** *11*, 161-163.
[7] MM Karthikeyan; G Ananthan; Jaffar Ali A. *Global veterinary*, **2010,** *4*, 255-259.
[8] K Ravinder; A Vijender Reddy; P Ramesh; S Ramakrishna; H Laatsch; Venkateswarlu Y. **2005**.
[9] VK Meenakshi; S Gomathy; Chamundeswari KP. *International Journal of ChemTech Research*, **2012,** *4*, 55-62.
[10] VK Meenakshi; S Gomathy; M Paripooranaselvi; Chamundeswari KP. *International Journal of Chemical and Pharmaceutical Sciences*, **2012,** *3(2)*, 33-39.
[11] VK Meenakshi. Biology of a few chosen ascidians, Ph.D., Thesis, Manonmaniam Sundaranar University, (Tirunelveli, Tamilnadu, India, **1997**).

[12] WC Evans. Trease and Evans' Pharmacognosy, 14th ed., WB Saunders company, Singapore, **1997**; pp. 441-450.
[13] MN Chatterjea; Rana Shinde, Text book of Medical Biochemistry, 2nd ed., Jaypee Brothers Medical Publishers (P) Ltd, New Delhi, **1995**; pp. 190-193.
[14] Gelinas, Pierre; McKinnon, M Carole. *International Journal of Food Science and Technology*., **2006,** *41(3),* 329.
[15] P Valentão; E Fernandes; F Carvalho; PB Andrade; RM Seabra; ML Bastos. *Journal of Agricultural and Food Chemistry*, **2001,** *49(7),* 3476-3479.
[16] E Graf. *Free Radical Biology and Medicine*, **1992**, *13(4),* 435-448.
[17] SM Fiuza; C Gomes; LJ Teixeira; MT Girão da Cruz; MNDS Cordeiro; N Milhazes; F. Borges; M.P.M. Marques. *Bioorganic and Medicinal Chemistry*, **2004,** *12(13),* 3581-3589.
[18] MK Rasool; EP Sabina; SR Ramya; P Preety; S Patel; N Mandal; PP Mishra; J Samuel. *Journal of Pharmacy and Pharmacology*, **2010,** *62(5),* 638-643.
[19] Ren-Wang Jiang; Kit-Man Lau; Po-Ming Hon; CW Thomas Mak; Kam-Sang Woo; Kwok-Pui Fung. *Current Medicinal Chemistry*, **2005,** 12(2), 237-246.
[20] MR Cesarone; G Laurora; A Ricci; G Belcaco; P Pomante. *Journal of Vascular Disease*, **1992,** *21*, 76-80.
[21] W Clack; W Heller; C Michel; M Saran. *Journal of Allergy*, **1950**, *21*, 133-147.
[22] PO Colergie Smith; P Thomas; JH Scurr; JA Dormandy. *British Medical Journal*, **1980,** *296*, 172-176.
[23] MGL Hertog; PCH Hollman; MB Katan; M Klohout. *Nutrition and Cancer an International Journal*, **1993**, *20*, 21-29.
[24] C De-whalley; SM Rankin; JRS Houct; W Jessup; DS Leake. *Biochemical Pharmacology*, **1990**, *39*, 1743-1750.
[25] RF Vitor; H Mota-Filipe; G Teixeira; C Borges; AI Rodrigues; A Teixeira; A Paulo. *Journal of Ethnopharmacology*, **2004,** *93(2-3),* 363-370.
[26] S Nees; DR Weiss; E Reichenbach-Klinke; F Rampp; B Heilmeier; J Kanbach; A Esperester. *Arzneimittel-Forschung/Drug.* **2003,** *53(5),* 330-341.
[27] YQ Li; FC Zhou; F Gao; JS Bian; F Shan. *Journal of Agricultural and Food Chemistry*, **2009**, *57*, 11463-11468.
[28] BA Silva, O Malva, AC Dias. *St. John's Wort and Food Chemistry*, **2008,** *110*, 611-619.
[29] Rui Zhang; Yang Yao; Yingping Wang; Guixing Ren. *Nutrition and Metabolism* 2**011**, *8*, 85.