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Isolation and identification of flavonoid in *Strychnos nux-vomica* L., and evaluation of antioxidant potential

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Abstract
In the present work, methanolic extract from the leaves of *Strychnos nux-vomica* were analyzed phytochemically for the presence of flavonoids. Phytochemical studies revealed the presence of flavonoidal structure, by using chromatographic and spectroscopic techniques 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside i.e., rutin is identified. Antioxidant potential was determined by DPPH method.

Keywords: *Strychnos nux-vomica,* flavonoids, IR, NMR, antioxidant potential, DPPH method

Introduction
The medical attribution of *Strychnos nux-vomica* in Indian system of medicine has been known for a long time. Since the time of Sushruta it is used in Ayurvedic medicine. It belongs to family Loganiaceae is an indigenous tree of medicinal importance used in Ayurveda. It is a medium sized tree, found throughout tropical India, Bihar, Orissa, Uttar Pradesh, up to altitudes of 360 m in Sahyadri foot hills of Western Ghats. Leaves 7.5-15 cm long, broadly elliptic, acute, obtuse or shortly acuminate, glabrous, base usually rounded. In English it is commonly known as Snake wood, Poison nut, Kuchla in Hindi, Kajra in Marathi, Kunchila in Bengali, Kaajjeel in Malayalam, Ajaraki in Arabian etc. *Strychnos nux-vomica* exhibits antitumor, antimicrobial, anticonvulsion, antiamnesic, immunomodulatory effects [1].

Free radicals represent an essential part of aerobic life and metabolism [2] and are principle to anti-biochemical process. Due to their roles of combating myriads of oxidative damages incurred by living cells from free radical activities, over the past decades natural antioxidants of both nutritive and medicinal plants have been of significant interest to the pharmaceutical and food industries. By scavenging the free radicals, antioxidants offer resistance against oxidative stress and by many other mechanisms and thus prevent the disease progression [3]. The most commonly used synthetic antioxidants at present are butylated hydroxyl anisole (BHA), propyl gallate, and tertbutyl hydroquinone. However they are suspected of being responsible for liver damage and acting as carcinogens [4]. The search for new products with anti-oxidative properties and fewer side effects is very active domain of research. Therefore, the development and utilization of more effective antioxidants of natural origin is desirable [5]. Since ancient times, many official herbs have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of any infection and preservation of food from toxic effects of oxidants [6]. Novel natural antioxidants from some plants have been extensively studied in the past few years for their antioxidants and radical scavenging properties.

Over 8000 structurally remarkable flavonoids have been found in plants. These compounds have ordinary features i.e., phenyl benzopyrone skeleton (C_6-C_3-C_6). Flavonoids are among the most potent natural antioxidants, and many of them are stronger reducing agents on a molar basis than ascorbic acid [7-8]. On the aromatic rings, the number and position of hydroxyl groups determine the antioxidant capacity of polyphenol's. Flavones and their derivatives were found to be the most effective antioxidants during a comparative study among different flavonoid structures [9].

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. For protecting the body against reactive oxygen species, the flavones and catechins seem to be the most powerful flavonoids. Body cells and tissues are continuously threatened by the damage caused by free radicals and reactive oxygen species, which are

produced during normal oxygen metabolism or are induced by exogenous damage [10-11]. The mechanisms and the sequence of events by which free radicals interfere with cellular functions are not fully understood, but one of the most important events seems to be lipid peroxidation, which results in cellular membrane damage. This cellular damage causes a shift in the net charge of the cell, changing the osmotic pressure, leading to swelling and eventually cell death. Free radicals can attract various inflammatory mediators, contributing to a general inflammatory response and tissue damage. To protect themselves from reactive oxygen species, living organisms have developed several effective mechanisms [12]. The antioxidant-defense mechanisms of the body include enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, but also nonenzymatic counterparts such as glutathione, ascorbic acid, and α-tocopherol. The increased production of reactive oxygen species during injury results in consumption and depletion of the endogenous scavenging compounds. Flavonoids may have an additive effect to the endogenous scavenging compounds. Flavonoids as antioxidants can prevent carcinogenesis [13].

Antioxidants are scavengers of oxygen radicals or hydroxy radicals that attack polyunsaturated fatty acids in cell membranes giving rise to lipid peroxidation. Lipid peroxidation is strongly associated with aging and carcinogenesis. Many natural resources have been tested for their antioxidant effect and some of them accumulated large amounts of C-glycosylflavones.

Dietary flavonoids are natural antioxidants [14]. Flavonoids are good antioxidants because of more number of target sites for free radicals [15] Flavonoids have existed for over one billion years and possess antioxidant [16], anti-thrombic [17], anti-hypertensive [18], anti-inflammatory [19] activity. Flavonoid serves as antioxidant by scavenging singlet oxygen [20], lipid peroxy radicals [21] and superoxide anion [22].

The main object of this study is to extract and characterize flavonoid antioxidant in the leaves of *Strychnos nux-vomica.*

Experimental

^{1}H NMR and ^{13}CNMR spectra were recorded on a Bruker Advance 400 MHz spectrometer. The EI-mass was recorded on Shimadju QP 2000 mass spectrometer. The FT-IR spectrum was recorded on Perkin Elmer, spectrum100 instrument. UV-spectra were recorded on Shimadju UV-160 spectrophotometer. The leaves of *Strychnos nux-vomica* was washed thoroughly with tap water followed by rinsing with double distilled water and shade drying for fifteen days. The fine powder was obtained from dried leaves by using kitchen mixer grinder (Philips electronics). The leaves powder was sterilized at 120^{o} C for 15 minutes. The leaves powder was stored under dessicator for further studies. Solvent extraction of dried powder (25gm) of *Strychnos nux-vomica* was done using 250ml. of 80% methanol in a soxlet extractor for 36 hours. The extract was concentrated by evaporation (40^{o}-50^{o}) in vaccum rotatory. The concentrated methanolic extract (10ml.) was suspended in 50ml. of distilled water and was further extracted twice with hexane and then with ethyl acetate. The ethyl acetated fractions were washed two times with distilled water. The ethyl acetate fraction was analyzed for flavonid using chromatographic separation. The glass plates (20x20cm) coated with silica gel (0.2- 0.3mm) were dried naturally (atmosphere). Subsequently they were activated at 100^{o}C for 30 minutes and were cooled at temperature 25^{o}C. Diluted samples of leaves of *Strychnos nux-vomica* were qualitatively studied by TLC, butanol: acetic acid: water (4:1:5) upper layer was used as mobile phase. TLC plates coated with silica gel were used as stationary phase. The plates were sprayed with a solution of 1% ethanolic 2-amino ethyl diphenyl borinate followed by 5% ethanolic solution of polyethylene glycol-400. Flavonoid appears in color zone under UV-365nm. Standard flavonoids were used for identification. Retention time is 0.42. With reagent A fluorescent yellow color and with reagent B orange color is obtained. The remaining extract was evaporated and residue was obtained, it was subjected to various physical and spectral analyses.

Chemical identification of flavonoids [23]

1. Alkaline reagent test: - To the test solution on addition of few drops of sodium hydroxide, intense color was formed which turn colorless on the addition of dilute acid.
2. Shinoda Test: -To a small amount of test solution in alcohol, magnesium ribbon was added followed by addition of drops of concentrated hydrochloric acid; formation of pink color confirms the presence of flavonoids.
3. Aluminum Chloride Test: - To a small amount of test solution, two drops of 1% aluminum chloride was added, yellow color was obtained.
4. Zn- Hydrochloride Reduction Test: - To the test solution add a mixture of zinc dust and concentrated hydrochloric acid. Heat the solution, after few minutes, color of the solution changes to red.
5. Test solution (0.5gm) was dissolved in 5ml of distilled water and filtered. To 1ml of the extract filtrate; 5ml dilute ammonia was added. 1ml concentrated sulphuric acid was added. On standing yellow coloration disappears confirms the presence of flavonoids.

Rutin, light yellow powder, m.p 190^{o}C, molecular formula $C_{27}H_{30}O_{16.}$ IR (KBr) V_{max}cm-^{1}: 3410, 3322(OH-stretching), 2842(CH_2-stretching), 2715 (C-H bonding), 1462 (C=O groups) and 1384 (C-OH vibrations).

^{1}HNMR(300 MHz, CD_3OD) δppm = 6.22(*d, J=2.0* HZ, 1H, H-6),6.38(*d, J*=2.0Hz,1H,H-8), 7.68 (*d,J*=2.05Hz, 1H, H-2'), 6.88 (*d, J*=8Hz, 1H, H-5'),7.64 (*dd, J*=8.5 & 2.2Hz, 1H, H-6'), 5.10(*d, J*=7.5Hz,1H,H-1"),4.54(*d,J*=1.5Hz,1H,H-1"'),1.10(*d,J*=6.3Hz,3H,H-6"'),3.30-3.86(m). ^{13}CNMR(75.5 MHz, CD_3OD) δppm=158.7 (C-2),135.7 (C-3), 179.6(C-4),163.2(C-5,)100.3(C-6),166.2(C-7),95.1(C-8),159.5(C-9,)105.9(C-10,)123.3(C-1'),117.8(C-2'),145.9(C-3'),149.9(C-4')116.2(C-5'),123.6(C-6'),104.9(C-1'')75.8(C-2''), 78.2 (C-3''),71.4 (C-4''),78.0 (C-5'') 68.8 (C-6''),102.4 (C-1'''),72.2 (C-2'''),72.3 (C-3''')74.1 (C-4'''),69.8 (C-5'''),18.1 (C-6''')

Antioxidant potential

To determine antioxidant activitiy 3.96 mg of 1,1-diphenyl-2-picrylhydrazyl was dissolved in 20ml of methanol to make a stock solution. 5gm leaves of *Strychnos nux-vomica* was extracted with 50ml methanol for 5 days with shaking at regular interval. The extract was filtered and concentrated by vaccum rotatory evaporator. 5mg of this extract was dissolved in 20ml methanol to make a stock solution. 0.5ml of sample solution was added to 1ml of DPPH solution separately. These solutions were incubated for 30 minutes at room temperature in dark. The absorbance was measured at 517 nm. Lower absorbance of the solution indicated higher free radical scavenging activity by equation:

Percentage of scavenging DPPH free radical = 100 X (1-AE/AD)

Where AE is absorbance of the sample solution and AD is the absorbance of the DPPH solution with nothing added.

Result and Discussion

The UV spectrum of this compound exhibited two major absorption peaks in the region 355nm and 258nm, which indicates the presence of flavonol structure. Mass spectra of isolated compound show molecular ion m/z 610 [M^+] corresponding to the molecular formula $C_{27}H_{30}O_{16}$. IR spectra showed OH-stretching at 3410 cm^{-1}, 3322cm^{-1}, CH_2-stretching at 2842cm^{-1}, CH-bonding at 2715 cm^{-1}, CO group at 1462 cm^{-1}, C-OH vibration at 1384 cm^{-1}. The ^{1}HNMR spectrum showed a two doublet proton at the region δ6.22 and δ6.38 corresponding to H-6 and H-8 protons respectively. The protons at C-6 and C-8 of flavonols which contain the common 5, 7-dihydroxy substitution pattern give rise to two doublets in the range δ6.0- δ6.5ppm. The H-6 doublet occur consistently at higher field than the signal for the H-8 [24]. Four doublets proton occurs at δ4.54, δ5.10, δ6.88 and δ7.68 corresponding to H-1’’’, H-1’’, H- 5’ and H-2’protons respectively. One double doublet proton found at the region δ 7.64 corresponding to H-6’ proton. ^{13}CNMR showed 27 carbon signals, double bond between C-2 and C-3. The carbonyl carbon appears at δ179.6. The carbonyl carbon resonates at around δ175- δ178 ppm, when the carbonyl is not hydrogen bonded, but in the presence of hydrogen bonding to a 5-hydroxyl group it moves downfield to about δ182ppm [25].

In this study antioxidant potential of the leaves of *Strychnos nux-vomica* was determined using DPPH method. At room temperature DPPH is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule. The decrease in DPPH absorbance at 517nm induced by antioxidants shows its reduction capability. The antioxidants causes decrease in absorbance of DPPH radical because when reaction between antioxidant molecules and radicals occurs then it causes scavenging of the radical by hydrogen donation. It is visually noticeable as a change in color from purple to red. The free radical scavenging activity of leaves of *Strychnos nux-vomica* is 61.25%.

From the physical, chemical and spectral characteristics it was confirmed that the isolated compound was 3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside i.e., rutin. The rutin has been earlier reported in other plants [26].

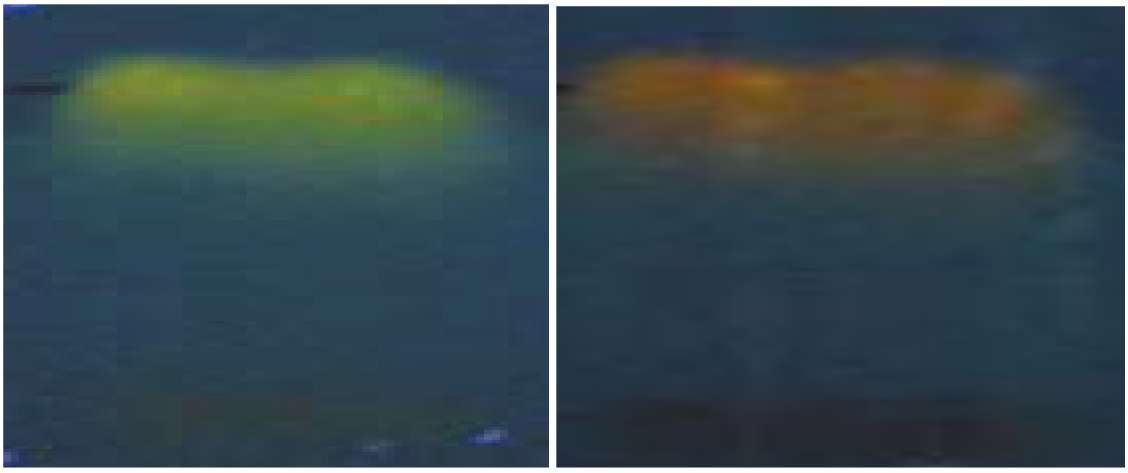

Fig 1: Flavonoid finger printing of rutin in the leaves of *Strychnos nux-vomica*

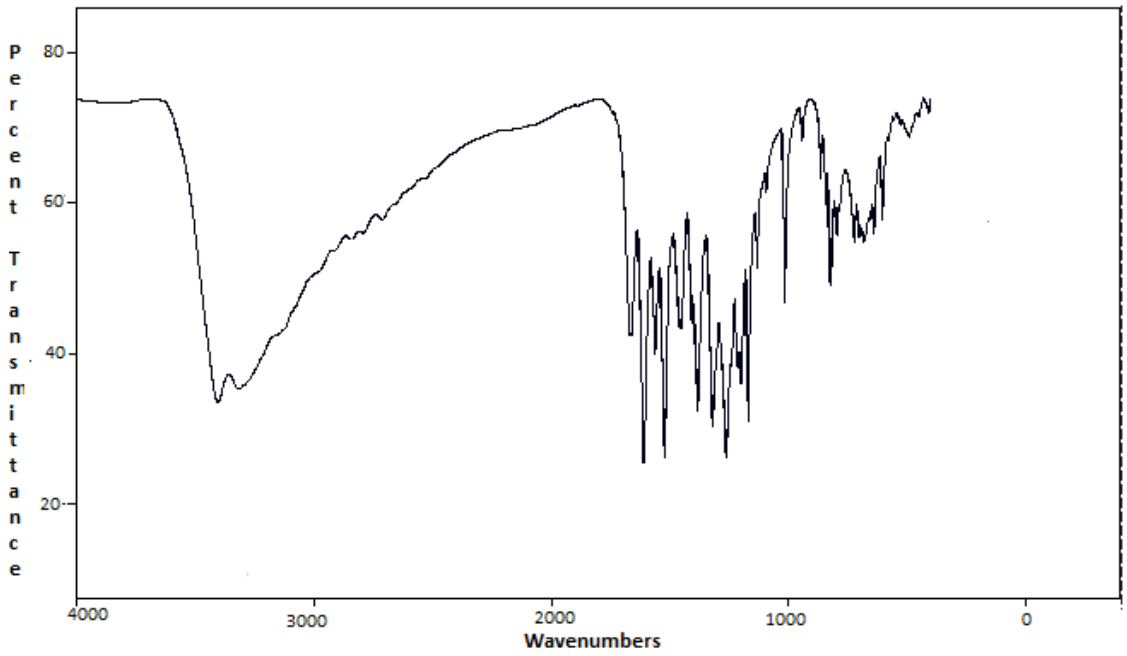

Fig 2: I R of Isolated sample

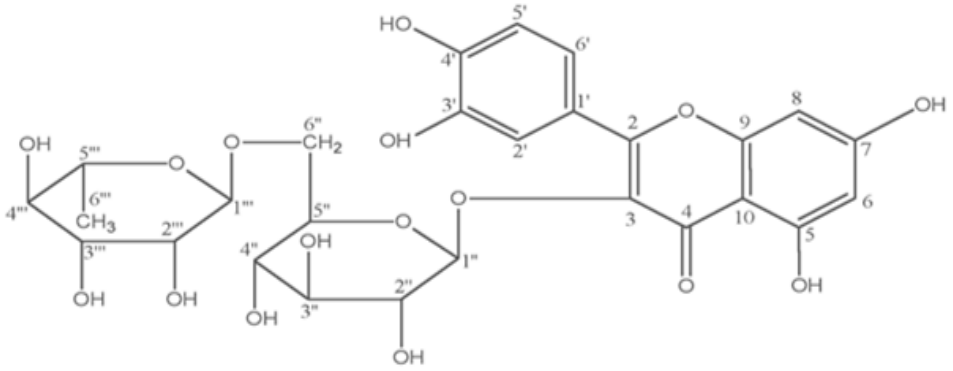

Fig 3: Structure of Rutin

Conclusion

Rutin (3, 3′, 4′, 5, 7-pentahydroxyflavone-3-rhamnoglucoside) is an important flavonoid that is consumed in the daily diet thus rutin is an important dietary constituent of food [27-30]. It is also known as vitamin P and quercetin-3-*O*-rutinoside. Rutin possess antioxidant activity, prevented low- density protein (LDL) peroxidation and the Fenton reaction [31-34]. Rutin is a promising flavonoid for lowering the risk of atherosclerosis due to its inhibiting on LDL oxidation. Rutin has antiviral, antihypertensive and antiplatelet properties, due to its high radical scavenging activity and antioxidant capacity it strengthens the capillaries so it is used in the treatment of various conditions related to capillary bleeding and increased capillary fragility and permeability.

Rutin can reduce capillary fragility, swelling and bruising and has been used in the treatment of venous insufficiency (varicose veins, haemorrhoids, diabetic vascular disease, and diabetic retinopathy), and for improving micro-vascular blood flow (pain, tired legs, night cramps, and restless legs) [35].

In addition, it is found in many food items, vegetables, and beverages. The cytoprotective effects of rutin, including gastro protective, hepatoprotective, and anti-diabetic effects, have been shown in several studies. Furthermore, rutin has several pharmacological effects such as anti-inflammatory and anti-glycation activities. Rutin protects against DNA damage [36]. Rutin is protective against carcinogensis [37]. Flavonoids are a part of human diet. *Strychnos nux-vomica* contains rutin which is a flavonoid. The production of rutin from *Strychnos nux-vomica* may be of economic benefit. This study confirms flavonoid rutin in the leaves of *Strychnos nux-vomica* which have free radical scavenging activity due to which leaves of this plant possess antioxidant properties.

Conflict of Interest statement

We decline that we have no conflict of interest.

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References

1. Kumar Arun M, Subrat J, Nisha O, Abhimanyu KA. Comprehensive review on effects of sodhan karma (Detoxification procture) and therapeutic potential of visha- tinduka (*Strychnos nux-vomica*). Int. J Res Ayurveda Pharm. 2012; 3:211-213.
2. Tiwari A. Imbalance in antioxidant defense and human disease: multiple approach of natural antioxidants therapy. Curr. Sci. 2001; 81:1179-1187.
3. Brauggler JM, Duncan LR, Chase. The involvement of iron III lipid peroxidation: importance of ferrous to ferric ratio in initiation. J Biol Chem. 1999; 61:102-182.
4. Anagnostopoulou, Kefelas P, Papageorgiou D. Radical scavenging activity of various extracts and fractions of sweet orange peel *(Citrus sinensis).*Food Chem. 2006; 94:19-25.
5. Sagakami H, Achi T, Simpson A, Tanuma SC. Induction of irr. munopotentiation activity by a protein - bound polysaccharide PSK. Anticancer Res 1999; 1:993-1000.
6. Tatjana KP, Svetlana K, Marina S. Invitro antioxidant activity of some Teucrium species (Lamiaceae). Acta Pharm 2005; 55:207-214.
7. Lee KW, Kim YJ, Kim DO, Lee HJ, Lee CY. Major phenolics in apple and their contribution to the total antioxidant capacity. J Agric Food Chem. 2003; 51:6516-6520.
8. Rice-Evans CA, Miller NJ, Pagangu G. Antioxidant properties of phenolic compounds. Trends Plant Sci.1997; 2:152-159.
9. Matsuda H, Morikawa T, Toguchida I, Yoshikawa M. Structural requirements of flavonoids and related compounds for aldose reductase inhibitory activity. Chem Pharm Bull. 2002; 50:788-795.
10. De Groot H. Reactive oxygen species in tissue injury. Hepatogastroenterology 1994; 41:328-332.
11. Grace PA. Ischaemia-reperfusion injury. Br J Surg. 1994; 81:637-47.
12. Halliwell B. How to characterize an antioxidant: an update. Biochem Soc Symp 1995; 61:73-101.
13. Stefani ED, Boffetta P, Deneo-Pellegrini H *et al*. Dietary antioxidants and lung cancer risk: a case-control study in Uruguay. Nutr Cancer 1999; 34:100-110.
14. Lewis NG. Plant Phenolics. In: antioxidants in higher plants by RG Alscher and J Hess (Eds) CRC Press, Boca Raton FL. 1993, 135-169.
15. Bors W, Michael C. Antioxidant capacity of flavonols and gallate esters: pulse radiolysis studies.Free Radic Biol Med. 1999; 27(11-12):1413-1426.
16. Belinky PA, Aviram M, Mahmood S, Vaya J. Structural aspects of the inhibitory effect of galbridin on LDL oxidation. Free Radic Biol Med. 1998; 24(9):1419-1429.

17. Shen JG, Zhou DY. Efficiency of Ginkgo biloba extract (EGB761) in antioxidant protection myocardial ischemia and reperfusion injury. Biochem Mol Biol Int. 1995; 35(1):125-134.
18. Liu X, Wei J, Tan F, Zhou S, Wurthwein G, Rohdewald P *et al.* French Maritime pine bark extract, improves endothelial function of hypertensive patients. Life Sci. 2004; 74(7):855-862.
19. Read MA. Flavonoids: naturally occuring anti-inflammatory agents. Am J Pathol. 1995; 147(2):235-237.
20. Hussain SR, Gillard J, Gillard P. Hydroxyl radical scavenging activity of flavonoids. Phytochemistry 1987; 26(9):2489-2491.
21. Torel J, Cillard J, Cillard P. Antioxidant activity of flavonoids and reactivity with peroxy radicals. Phytochemistry 1986; 25:383-385.
22. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol. 1988; 37(5):83-88.
23. Harborne JB. Phytochemical Methods. A guide to modern techniques of plant analysis. IIIrd ed., London, Chapmann and Hall, 1984.
24. Marby TJ, Markham KR, Thomas MMB. The systematic identification of flavonoids. Springler-Verglar Publishers: New York 1970, 1-58.
25. Markham KR, Chari VM. Carbon-13 NMR spectroscopy of flavonoids. In the flavonoids: Advances in research; Harborne JB, Marby TJ. Eds Chapmann and Hall; London United Kingdom 1982, 19-135.
26. Sampath M, Vasanthi M. Isolation, structural elucidation of flavonoids from *Polyalthia longifolia (Sonn.) Thawaites* and evaluation of antibacterial, antioxidant and anticancer potential. International Journal of Pharmacy and Pharmaceutical Sciences. 2013; 5(1):336-341.
27. Fabjan N, Rode J, Kosir IJ, Wang Z, Zhang Z, Kreft I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. J Agric Food Chem. 2003; 51:6452-6455.
28. Kreft S, Knapp M, Kreft I. Extraction of rutin from buckwheat (*Fagopyrum esculentum* Moench) seeds and determination by capillary electrophoresis. J Agric Food Chem. 1999; 47:649-4652.
29. Kuntic V, Pejic N, Ivkovic B *et al.* Isocratic RP-HPLC method for rutin determination in solid oral dosage forms. J Pharm Biomed Anal. 2007; 43:718-721.
30. Gulpinar AR, Orhan IE, Kan A, Senol FS, Celik SA, Kartal M. Estimation of in vitro neuroprotective properties and quantification of rutin and fatty acids in buckwheat (Fagopyrum esculentum Moench) cultivated in Turkey. Food Res Int. 2012; 46:536-543.
31. Caillet S, Yu H, Lessard S, Lamoureux G, Ajdukovic D, Lacroix M. Fenton reaction applied for screening natural antioxidants. Food Chem 2007; 100:542-552.
32. Katsube T, Imawaka N, Kawano Y, Yamazaki Y, Shiwaku K, Yamane Y. Antioxidant flavonol glycosides in mulberry (*Morus alba* L.) leaves isolated based on LDL antioxidant activity. Food Chem 2006; 97:25-31.
33. Ziaee A, Zamansoltani F, Nassiri-Asl M, Abbasi E. Effects of rutin on lipid profile in hypercholesterolaemic rats. Basic Clin Pharmacol Toxicol. 2009; 104:253-258.
34. Jiang P, Biuarczynski F, Campbell C, Pierce G, Austria JA, Brigg CJ. Rutin and flavonoid contents in three buck wheat species Fagopyrum esculentum, F. tataricimand F. homotropicum and their protective effect against lipid peroxidation. Food Res Int. 2007; 40:356-364.
35. Grinberg LN, Rachmilewitz EA, Newmark H. Protective effects of rutin against hemoglobin oxidation. Biochem. Pharmacol. 1994; 48:643-649.
36. Zhao CY, Shi YM Wang WF, Jia ZJ, Yao SD, Fan BT. Fast repair of deoxythymidine radical anions by two polyphenols: rutin and quercetin. Biochem Pharmacol. 2003; 65:1967-1971.
37. Webster RP, Gawde MD, Bhattachary RK. Protective effect of rutin, a flavonol glycoside on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer Lett. 1996; 109:185-191.