Original Article

Experimental studies on bioactive potential of rutin

Abstract

Background: Plant-derived phytochemicals are gaining wide popularity owing to their diverse therapeutic potential and less side effects. Rutin is one of the plant-derived flavonoid. Rutin has demonstrated cardio protective, analgesic, and anticancer effects. **Aim:** The current work was focused to evaluate bioactive potential of rutin. **Materials and Methods:** Rutin was isolated from tobacco leaves. The structure was confirmed by H^1 NMR spectroscopy. The isolated rutin was studied for possible antibacterial, antifungal, anthelmintic, larvicidal, and cytotoxic effects. **Results:** Results of studies demonstrated that rutin effectively inhibited growth of bacteria and fungi, as well as demonstrated anthelmintic potential. There was a positive response for larvicidal and cytotoxic effects. **Conclusion:** These studies justify chemotherapeutic potential of rutin.

Key words:
Anthelmintic, antibacterial, antifungal, cytotoxic, larvicidal, rutin

Introduction

The infections caused any means either by parasite, microbes, or funguses are one of the major health problems in all ages. Inadequate sanitation, hygiene, and untidy environmental conditions are the probable reason suggested for it.[1] Parasitic nematodes affect considerably all age groups, causing ill health and poor growth with a number of diseases like helmintic infections of gastro intestinal tract (resulting in dyspepsia, constipation, and other conditions) and malaria. Malaria is now a major health problem, especially in sub-tropical and tropical countries. According to the estimation of WHO, about 41% of the world's total population resides in areas with parasitic risk.[2,3] In the present era, many modern drugs are originated from plant origin; it is now necessary that other medicinal plants that exhibit folklore reputation for antimicrobial and antiparasitic properties be investigated for their safety, efficacy, and potential as sources of new drugs.[4] The drugs used as anthelmintics and larvicidal to treat and control infections are expected to produce toxic effects on them. With the increased use of synthetic compounds, parasitic resistance has emerged.[5] Presently, multi-drug resistance has become a major issue.[6,7] The problem arising due to drug resistance and side effects caused by synthetic drugs have focused the modern research toward natural remedies. Flavonoids have served in treating the gastrointestinal tract-related disorders along with medicinal properties like laxative and anthelmintic, and are effective in leprosy, ringworm infestation, and also in flatulence, colic, dyspepsia, constipation, cough, bronchitis, and cardiac disorders.[8,9] Many plants contain flavonoids that have been previously reported to have anticancer properties.[10,11] The plants flavonoids have been found effective against various parasitic infestations.[12] Hence, the aim of the present study was to screen antibacterial, antifungal, cytotoxic, anthelmintic, and larvicidal properties of rutin.

Access this article online	
Website: www.cysonline.org	Quick Response Code
DOI: 10.4103/2229-5186.115556	

Materials and Methods

Chemicals

Until otherwise specified, all chemicals were purchased from CDH, India.

Test organisms

Test microbes

Bacterial and fungal cultures used in the present studies were obtained from Microbial Type Culture Collection (MTCC)

Shagun Dubey, Aditya Ganeshpurkar, Divya Bansal[1], Nazneen Dubey

Drug Discovery Laboratory, [1]Pharmaceutics Research Laboratory, Shri Ram Institute of Technology-Pharmacy, Jabalpur, Madhya Pradesh, India

Address for correspondence:
Asst. Prof. Aditya Ganeshpurkar,
Shri Ram Institute of Technology-Pharmacy,
Jabalpur - 482 002, Madhya Pradesh, India.
E-mail: adityaganeshpurkar@gmail.com

IMTECH, Chandigarh. The bacterial strains used were *Escherichia coil* MTCC 2960, *Pseudomonas auruginosssa* MTCC 4676, *Staphylococcus aureus* MTCC 3160, *Klebsiella oxytoca* MTCC 3030, *Bacillus subtilis* MTCC 1790, *Candida albicans* MTCC 183.

Test worms

The roundworms (*Ascaridia galli*) were obtained from intestine of freshly slaughtered fowls. Earthworms (*Pheretima phostuma*) (0.8-2 g) were collected from the local gardens of Jabalpur (Madhya Pradesh), India.

Test mosquitoes larvae

The present study was conducted at Jabalpur (23.1700°N, 79.9500°E), Madhya Pradesh, India, during September to October, 2012. Larvae of *S. aegypti* were maintained at 25-30°C. The larvae were fed on a powdered mixture of dog biscuits and dried yeast powder at a ratio of 3:1. The adult colony was provided with 10% sucrose solution and 10% multivitamin syrup.

Cells for tetrazolium salt assay

The leukocytes were obtained from human peripheral blood, incremented with heparin, and centrifuged with Phycoll isopaque (Histopaque-1077).

Isolation of flavonoids

The isolation was performed as detailed by Zhang *et al*.[13] Tobacco leaves were dried and about 50 g of the powder was placed in a reflux apparatus. The extraction was performed at 80°C for 60 min with 85% (v/v) aqueous ethanol. The filtered solution was concentrated under reduced pressure. The purification of crude flavonoids-enriched extract was done via column (25 × 1.5 cm^2), which was packed with AB-8 macroporous adsorption resin. The conditions followed were injecting concentration 3.75 mg/ml, pH 5, injecting velocity 2.0 ml/min, 40% (v/v) ethanol as desorption solvent, and desorption velocity of flow 1.5 ml/min. The extract was collected and was evaporated at 50°, followed by freeze drying. H^1 NMR studies were performed to confirm its structure.

Antimicrobial studies

Antibacterial and antifungal studies

The stock solution of rutin was prepared in dimethylsulphoxide (DMSO) at a concentration of 1 mg/ml. Ampicillin (25 µg) and Clotrimazole (30 µg) were used as reference agents for bacteria and fungi, respectively. The test for susceptibility was determined by disc diffusion method.[14] Nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min, 0.1% inoculum suspension was swabbed uniformly on them, and the inoculum was allowed to dry for 5 min. Rutin was loaded on 6-mm discs. The loaded discs were placed on the surface of medium, the extracts were allowed to diffuse for 5 min, and the plates were kept for incubation at 37°C for 24 h for bacteria and 30°C for 48 h for fungi with yeast peptone dextrose agar. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimeters.

Determination of minimal inhibitory concentration

MIC was determined was determined by broth dilution susceptibility assay.[15] For this assay, the bacterial strains were cultured overnight at 37°C in nutrient agar and *Candida albicans* was cultured overnight at 30°C in yeast peptone dextrose agar. Bacterial and fungal strains were suspended in their corresponding broths to give a final density of 106 and 105 organism/ml, respectively. Different dilutions of rutin 1, 2, 3, and 4 ranging from 10 µg/ml to 0.05 µg/ml were prepared in capped tubes. A control was also served, and 20 µl from each of the test organisms was used to inoculate the tubes. The tubes were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for fungi. Tubes containing broth (2 ml) were inoculated with organisms and kept at 4°C in a refrigerator overnight to be used as standards. The MIC was recorded as the lowest concentration at which no microbial growth was observed.

Anthelmintic bioassays

The anthelmintic study was carried as per Ajayieoba *et al*.[16] Both methanolic extract and its ethyl acetate fraction were dissolved in normal saline containing 5% DMF and diluted to get concentrations of 25, 50, 75, and 100 mg/ml. Piperazine (20 mg/ml) was used as the standard drug. All the solution used, i.e., drug and rutin, were prepared freshly. Eight groups were prepared with five earthworms each and each was placed in 10 ml of desired solution (normal saline containing 5% DMF), piperazine (20 mg/ml), and two sets of three different groups were treated with extracts of respective concentrations. The time of paralysis and time of death for each individual set was observed. The paralysis was defined as restricted movement of worms even in normal saline and death was understood as loss of motility followed by fading of body color.

Larvicial bioassays

The assay for larvicidal was carried out as per World Health Organization (WHO) standard protocols[17] with certain modifications. All the concentrations of rutin, i.e., 25, 50, 75, and 100 mg/ml, were transferred into sterile glass petri dishes in which larvae were introduced separately. The mortality data was recorded after 24, 48, and 72 h of the exposure. The death of larvae was said when they failed to move even after needle probing in cervical region. The experiment was repeated with similar concentration of parathion and different observations were made.

Cytotoxic studies (colorimetric MTT (tetrazolium) assay)

The MTT assay was done as per Mosmann *et al*.[18] Human peripheral blood was used as source of leukocytes, which incremented with heparin and centrifuged with Phycoll

isopaque (Histopaque-1077) in a 5% CO_2 atmosphere. The cells were washed successively with RPMI 1640 medium and supplemented with 50 µM 2-mercaptoethanol and 5-10% fetal bovine serum. A total of 100 µl of the solution was added to the cells in a ratio of 1 × 106 cells/well. These cells had been incubated for 4 h with different concentrations (0.5, 1.0, and 1.5 µg/ml) of rutin. Rutin and stock MTT solution (10 µl/100 µl medium RPMI) was added to all assay wells, and the plates were incubated at 37°C for 4 h. Acid-isopropanol (100 µl of 0.04 N HCl) was added to all the wells and mixed thoroughly to dissolve the dark blue crystals. The plates were read at 570 nm.

Statistical analysis

The results are expressed as mean ± standard error of mean. Experiments were always performed in triplicates. Statistical comparison was performed using analysis of variance (ANOVA) followed by Bonferroni's test (*P<0.001).

Results

In the current work, rutin was isolated and purified from tobacco; its structure was confirmed by H^1 NMR spectroscopy [Figure 1]. The results of antimicrobial studies revealed that rutin is good antibacterial and antifungal agent. Tables 1 and 2 summarize the results of antimicrobial effect of rutin on six microorganisms. Rutin showed antifungal activity against *C. albicans* and prominent bacterial activity against *S. aureus*, *B. subtilis, E. coli*, and *K. oxytoca*.

In the current work, both test (rutin) and standard showed a concentration-depended anthelmintic and larvicidal property. The anthelmintic drugs, like piperazine, act by causing paralysis of worms and they are expelled in feaces of man and animals. Rutin not only caused paralysis but also death of the worms. At concentrations 100 mg/ml, rutin had activity significantly (P<0.001) than the reference drug for all group of worms and larvae. Rutin had a concentration related activity, which was 2-4 times lower than that of the reference compound. The results were found significant at P<0.001 [Figure 2]. It is well known that anthelmintics act by paralyzing worms, which may have to be expelled by a purge. Similar results were obtained in larvicidal studies [Figure 3]. Rutin significantly (P<0.001) demonstrated larvicidal potential [Figure 3]. In the present study, MTT assay demonstrated significant (P<0.001) cytotoxic potential of rutin on isolated leucocytes [Figure 4].

Discussion

Nature has always served as an immense source for humans as it is known that the flavonoids have various properties like antimicrobial, fungicidal, anthelmintic, antimalarial, antidiabetic, leukemic, and larvicidal property. The results of the current study indicated various properties of rutin, and it was found that rutin is a potent antimicrobial, anthelmintic, larvicidal, and cytotoxic potential.

Rutin is one of the important phyto-antioxidant. One study demonstrated beneficial effect of rutin on CCl_4-induced hepatotoxicity in rats.[19] Oligorutin has also elicited

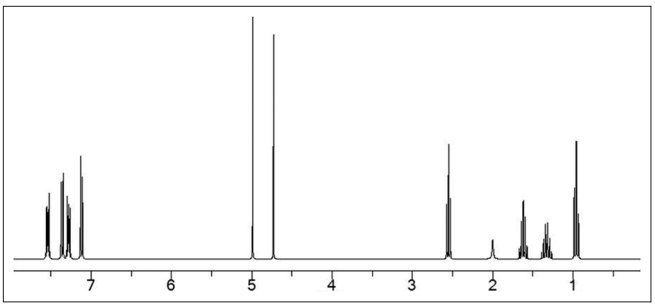

Figure 1: H1 NMR spectra of rutin

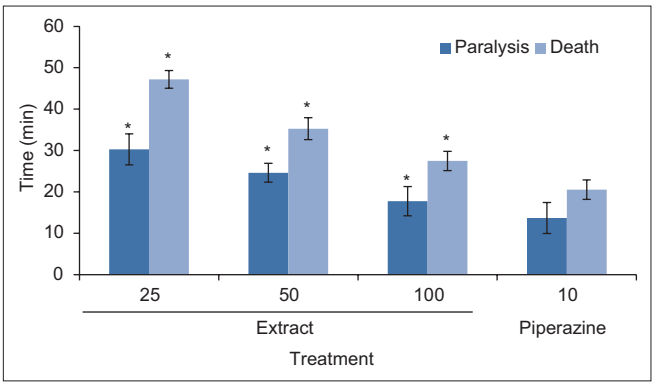

Figure 2: Anthelmintic activity of rutin. Each value is expressed as mean±SD; (n=3); *P<0.001

Table 1: Antimicrobial activity of rutin and antibiotic sensitivity of microorganisms (zone size, mm); Each value is expressed as mean±SD

Test organisms	Zone size (mm)		
	Rutin	Clotrimazole	Ampicillin
Staphylococcus aureus	15±1.7	–	21±1.5
Bacillus subtilis	7±0.9	–	8±0.7
Escherichia coli	9±0.8	–	9±0.7
Klebsiella oxytoca	13±1.3	–	5±0.3
Pseudomonas aurigenosa	12±0.8	–	9±0.7
Candida albicans	14±1.6	18±1.1	–

Table 2: Minimum inhibitory concentration of rutin

Test organisms	MIC (mm)		
	Rutin	Clotrimazole	Ampicillin
Staphylococcus aureus	6	–	4
Bacillus subtilis	5	–	4
Escherichia coli	7	–	3.5
Klebsiella oxytoca	8	–	2.4
Pseudomonas aurigenosa	12	–	5.8
Candida albicans	14	9	–

MIC – Minimum inhibitory concentration

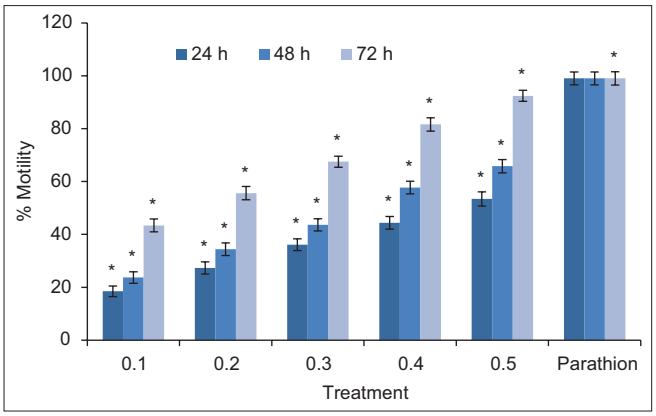

Figure 3: Larvicidal activity of rutin. Each value is expressed as mean±SD; (n=3); *P<0.001

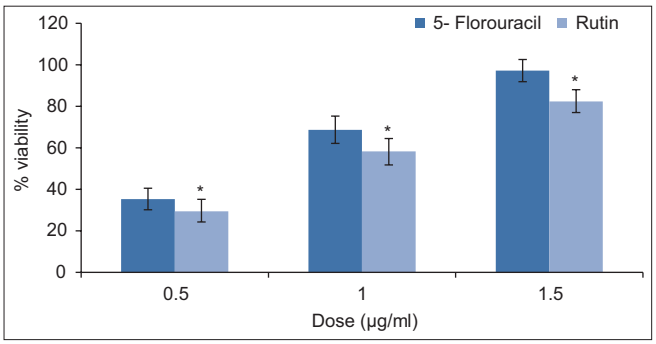

Figure 4: Effects of rutin on viability of human leucocytes. Each value is expressed as mean±SD; (n=3); *P<0.001

antimutagenic potential.[20] Rutin is known to show beneficial effect rutin on spatial memory as well as the concentration of brain neurotransmitters in aged rats.[21] Rutin has also shown antidiabetic potential in streptozotocin-induced diabetic istar rats.[22] It has also exerted protective effect on kidney during streptozotocin-induced diabetes[23] and in diabetic nephropathy.[24] Rutin has also demonstrated improvement in homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes.[25] Aging is also affected during diabetes by treatment of rutin.[26]

A number of plant extracts rich in flavonoids are known possess antimicrobial, anthelmintic, and cytotoxic potential.[27-36] The diverse therapeutic potential of plant flavonoids is due to their chemical structures. Such phytochemicals from plants serve as important therapeutic agents. A number of plants and macrofungi are known for therapeutic potential.[33,37-42]

The current work was focused to isolate rutin and to evaluate its chemotherapeutic potential. Rutin demonstrated antibacterial, antifungal, anthelmintic, larvicidal, and cytotoxic effects. The results of the studies are encouraging, but more systematic studies are required to confirm these effects.

References

1. Rehman T. Role of the gut microbiota in age-related chronic inflammation. Endocr Metab Immune Disord Drug Targets 2012;12:361-7.
2. Takesue A, Hiratsuka Y, Ono K, Murakami A. Towards the elimination of onchocerciasis at the global scale: History, challenges and future prospects. Nihon Ganka Gakkai Zasshi 2012;116:547-53.
3. Humphries D, Nguyen S, Boakye D, Wilson M, Cappello M. The promise and pitfalls of mass drug administration to control intestinal helminth infections. Curr Opin Infect Dis 2012;25:584-9.
4. Sutherland IA, Leathwick DM. Anthelmintic resistance in nematode parasites of cattle: A global issue? Trends Parasitol 2011;27:176-81.
5. Brooker S. Estimating the global distribution and disease burden of intestinal nematode infections: Adding up the numbers-A review. Int J Parasitol 2010;40:1137-44.
6. Vardakas KZ, Rafailidis PI, Konstantelias AA, Falagas ME. Predictors of mortality in patients with infections due to multi-drug resistant Gram negative bacteria: The study, the patient, the bug or the drug? J Infect 2013;66:401-14.
7. Karras G, Giannakaki V, Kotsis V, Miyakis S. Novel antimicrobial agents against multi-drug-resistant Gram-negative bacteria: An overview. Recent Pat Antiinfect Drug Discov 2012;7:175-81.
8. Falcone Ferreyra ML, Rius SP, Casati P. Flavonoids: Biosynthesis, biological functions, and biotechnological applications. Front Plant Sci 2012;3:222.
9. Mulvihill EE, Huff MW. Citrus flavonoids and the prevention of atherosclerosis. Cardiovasc Hematol Disord Drug Targets 2012;12:84-91.
10. Syed DN, Chamcheu JC, Mukhtar VM. Pomegranate extracts and cancer prevention: Molecular and cellular activities. Anticancer Agents Med Chem 2012.
11. Khan N, Syed DN, Ahmad N, Mukhtar H. Fisetin: A dietary antioxidant for health promotion. Antioxid Redox Signal 2012.
12. Prajapati RP, Kalariya M, Parmar SK, Sheth NR. Phytochemical and pharmacological review of Lagenaria sicereria. J Ayurveda Integr Med 2010;1:266-72.
13. Wang X, Wu Q, Wu Y, Chen G, Yue W, Liang Q. Response surface optimized ultrasonic-assisted extraction of flavonoids from Sparganii rhizoma and evaluation of their *in vitro* antioxidant activities. Molecules 2012;17:6769-83.
14. Selvaraj N, Lakshmanan B, Mazumder PM, Karuppasamy M, Jena SS, Pattnaik AK. Evaluation of wound healing and antimicrobial potentials of Ixora coccinea root extract. Asian Pac J Trop Med 2011;4:959-63.
15. Lalitha MK. Methods of Antimicrobial Susceptibility Testing. Manual Antimicrobial Susceptibility Testing. 2007. Available at http://www.ijmm.org/document/Antimicrobial.doc [Last accessed on 2009 Nov 15].
16. Nweze NE, Ogidi A, Ngongeh L. Anthelmintic potential of three plants used in Nigerian ethnoveterinary medicine. Pharm Biol 2013;51:311-5.
17. World Health Organization: Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. WHO/VBC; 1981, 81:807.
18. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
19. Khan RA, Khan MR, Sahreen S. CCl4-induced hepatotoxicity: Protective effect of rutin on p53, CYP2E1 and the antioxidative status in rat. BMC Complement Altern Med 2012;12:178.
20. Ben Rhouma G, Chebil L, Krifa M, Ghoul M, Chekir-Ghedira L. Evaluation of mutagenic and antimutagenic activities of oligorutin and oligoesculin. Food Chem 2012;135:1700-7.
21. Pyrzanowska J, Piechal A, Blecharz-Klin K, Joniec-Maciejak I, Zobel A, Widy-Tyszkiewicz E. Influence of long-term administration of rutin on spatial memory as well as the concentration of brain neurotransmitters in aged rats. Pharmacol Rep 2012;64:808-16.
22. Kamalakkannan N, Prince PS. Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin Pharmacol Toxicol 2006;98:97-103.
23. Kamalakkannan N, Prince PS. The influence of rutin on the extracellular matrix in streptozotocin-induced diabetic rat kidney. J Pharm Pharmacol 2006;8:1091-8.
24. Hao HH, Shao ZM, Tang DQ, Lu Q, Chen X, Yin XX, *et al*. Preventive effects

of rutin on the development of experimental diabetic nephropathy in rats. Life Sci 2012;91:959-67.

25. Stanley Mainzen Prince P, Kamalakkannan N. Rutin improves glucose homeostasis in streptozotocin diabetic tissues by altering glycolytic and gluconeogenic enzymes. J Biochem Mol Toxicol 2006;20:96-102.
26. Odetti PR, Borgoglio A, De Pascale A, Rolandi R, Adezati L. Prevention of diabetes-increased aging effect on rat collagen-linked fluorescence by aminoguanidine and rutin. Diabetes 1990;39:796-801.
27. Kosanić M, Ranković B. Antioxidant and antimicrobial properties of some lichens and their constituents. J Med Food 2011;14:1624-30.
28. Boligon AA, Sagrillo MR, Machado LF, de Souza Filho O, Machado MM, da Cruz IB, *et al*. Protective effects of extracts and flavonoids isolated from Scutia buxifolia Reissek against chromosome damage in human lymphocytes exposed to hydrogen peroxide. Molecules 2012;17:5757-69.
29. China R, Mukherjee S, Sen S, Bose S, Datta S, Koley H, *et al*. Antimicrobial activity of Sesbania grandiflora flower polyphenol extracts on some pathogenic bacteria and growth stimulatory effect on the probiotic organism Lactobacillus acidophilus. Microbiol Res 2012;167:500-6.
30. Bakar NS, Zin NM, Basri DF. Synergy of flavone with vancomycin and oxacillin against vancomycin-intermediate Staphyloccus aureus. Pak J Pharm Sci 2012;25:633-8.
31. Araruna MK, Brito SA, Morais-Braga MF, Santos KK, Souza TM, Leite TR, *et al*. Evaluation of antibiotic and antibiotic modifying activity of pilocarpine and rutin. Indian J Med Res 2012;135:252-4.
32. Thomsen H, Reider K, Franke K, Wessjohann LA, Keiser J, Dagne E, *et al.* Characterization of constituents and anthelmintic properties of Hagenia abyssinica. Sci Pharm 2012;80:433-46.
33. Ganeshpurkar A, Bhadoriya SS, Pardhi P, Rai G. Investigation of anthelmintic potential of oyster mushroom Pleurotus florida. Indian J Pharmacol 2012;44:539-40.
34. Lombardi G, Prosperini A, Font G, Ruiz MJ. Effect of polyphenols on enniatins-induced cytotoxic effects in mammalian cells. Toxicol Mech Methods 2012;22:687-95.
35. Boligon AA, Janovik V, Frohlich JK, Spader TB, Froeder AL, Alves SH, *et al*. Antimicrobial and cytotoxic activities of leaves, twigs and stem bark of Scutia buxifolia Reissek. Nat Prod Res 2012;26:939-44.
36. Marrassini C, Anesini C, Ferraro G. HPLC fingerprint of a flower extract of Tilia×viridis and correlation with antiproliferative and antioxidant activity. Phytother Res 2011;25:1466-71.
37. Bhadoriya SS, Mishra V, Raut S, Ganeshpurkar A, Jain SK. Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of *Tamarindus indica* leaves. Sci Pharm 2012;80:685-700.
38. Yadav P, Ganeshpurkar A, Rai G. *In vitro* H(+)-K(+) ATPase inhibitory potential of methanolic extract of *Cissus quadrangularis* Linn. Pharmacognosy Res 2012;4:123-6.
39. Bhadoriya SS, Ganeshpurkar A, Narwaria J, Rai G, Jain AP. *Tamarindus indica*: Extent of explored potential. Pharmacogn Rev 2011;5:73-81.
40. Gupta N, Ganeshpurkar A, Jatav N, Bansal D, Dubey N. *In vitro* prevention of chick pancreatic lipase activity by *Abroma augusta* extract. Asian Pac J Trop Med 2012;2:S712-5.
41. Ganeshpurkar A, Bhadoriya SS, Pardhi P, Jain AP, Rai G. *In vitro* prevention of cataract by Oyster Mushroom *Pleurotus florida* extract on isolated goat eye lens. Indian J Pharmacol 2011;43:667-70.
42. Ganeshpurkar A, Rai G, Jain AP. Medicinal mushrooms: Towards a new horizon. Pharmacogn Rev 2010;4:127-35.

How to cite this article: Dubey S, Ganeshpurkar A, Bansal D, Dubey N. Experimental studies on bioactive potential of rutin. Chron Young Sci 2013;4:153-7.

Source of Support: Authors duly acknowledge Reva Shiksha Samiti, Jabalpur, India (Support No.RSS/SRITP/MPY/113) for support during studies,
Conflict of Interest: None declared