British Journal of Pharmaceutical Research
9(4): 1-7, 2016, Article no.BJPR.21926
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Anti-bacterial and *in vitro* Anti-diabetic Potential of Novel Isoxazole Derivatives

Lincy Joseph[1*] and Mathew George[1]

[1]Pushpagiri College of Pharmacy, Kerala, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/21926
Editor(s):
(1) Hua Naranmandura, Department of Pharmacology, Toxicology and Biochemical Pharmaceutics,College of Pharmaceutical Sciences, Zhejiang University, China.
(2) Vasudevan Mani, Universiti Teknologi MARA (UiTM), Selangor, Malaysia.
(3) Ke-He Ruan,Director of the Center for Experimental Therapeutics and Pharmacoinformatics (CETP)Professor of Medicinal Chemistry & Pharmacology, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, USA.
Reviewers:
(1) Roukia Hammoudi, Université Kasdi Merbah,Ouargla, Algeria.
(2) Volodymyr Chernyshenko, NAS of Ukraine, Ukraine.
Complete Peer review History: http://sciencedomain.org/review-history/12300

Original Research Article

Received 9th September 2015
Accepted 29th October 2015
Published 16th November 2015

ABSTRACT

Aim: To synthesize novel Isoxazole derivatives, characterize them and subject for screening anti-bacterial action and *in vitro* anti-diabetic activity.
Methodology: Chalcones were prepared by the reaction of aromatic aldehydes with aromatic ketones in aqueous alcoholic alkaline medium. Then these were made to react with hydroxylamine hydrochloride and sodium acetate to prepare title compounds. The prepared isoxazole compounds were subjected to *in vitro* anti-diabetic screening by yeast and enzymatic method. All compounds were screened for antibacterial action by disc diffusion method.
Results: The structure of the synthesized compounds were confirmed by IR, NMR spectral datas and screened for anti-bacterial, and anti-diabetic activities. Most effective antibacterial one possessed chlorine in the Phenyl ring attached at 5-C of isoxazole and have NH_2 substitution in phenyl ring attached at 3-C of isoxazole. Compounds with significant *in vitro* anti-diabetic action by studying the glucose uptake by yeast cell method are with Br/NO_2 substituted forR_2/R_3 in phenyl ring when R'2 is OH/NH_2.
Conclusion: Presence of halogenated aromatic ring at 5-C and amine substituted phenyl ring at 3-Cof Isoxazole exhibited moderate anti-bacterial activity. In the anti-diabetic study halogenated or nitrated phenyl ring at 5-C and hydroxyl/amine substituted phenyl ring at 3-C of isoxazole exhibited anti-diabetic action.

**Corresponding author: E-mail: mathewlincg@yahoo.com;*

Keywords: Isoxazole; chalcones; aromatic aldehyde; aromatic ketones; antibacterial; in vitro anti-diabetic.

1. INTRODUCTION

Isoxazole being an azole with an oxygen atom next to the nitrogen, exhibits broad spectrum of biological activity and also forms a part of various biodynamic agents. Substituted isoxazoles are also considered to be important synthons due to their versatility towards chemical transformations to useful synthetic intermediates. A lot of modifications have been done during the last few years on isoxazole nucleus. A survey of literature revealed that substituted isoxazole possess different types of potent biological activities [1-3].

Many Isoxazole derivatives are stated to have good antimicrobial activity. Cali et al identified a series of isoxazole-3-hydroxamic acid derivatives as a new class of small, nonpeptidic inhibitors of peptide deformylase. They reported the synthesis, enzyme inhibition and preliminary investigation of the binding mode of this potential antibacterial compounds [4]. Antibacterial and antifungal studies done by Khanage et al. [5] revealed that compound containing -Cl, $-NO_2$ and $-OCH_3$ groups were found to be potent antimicrobial agents. Ravi et al. [6] synthesized novel methylene bridged benzisoxazolyl imidazo[2,1-b][1,3,4]thiadiazoles and reported antibacterial activity of synthesized compounds. Many studies indicate isoxazole derivatives improved diabetic condition. Kumar et al. [7] designed and synthesized a series of 3,5-diarylisoxazole derivatives as potential anti-hyperglycemic agents. Zhou et al. [8] synthesized fifteen new β-amino ketones containing a isoxazole moiety directly through Mannich reaction. They reported most of the compounds possess weak α-glucosidase inhibitory activity and protein tyrosine phosphatase 1B inhibitory activity in low concentration, some could activate peroxisome proliferator-activated receptors (PPAR) response element moderately [8]. In this light hereby prepared many Isoxazole derivatives which screened for anti-bacterial activity and *in vitro* antidiabetic activity.

2. METHODOLOGY

2.1 Preparation of Chalcones

0.01 mol of benzaldehyde was taken and added 0.01 mol acetophenone in 10 ml 95% ethanol in a flask. 3.5 mL 6 M NaOH solution was added to the reaction mixture stirred well for 10 minutes. Cooled in ice bath until crystal formation. 2 mL ice cold water added to it followed by 2 mL ice cold ethanol. Allow to air dry. Recrystallise from ethanol.

2.2 Cyclisation Step

The formed unstable chalcones were further cyclised with 0.015 mol of hydroxylamine hydrochloride and sodium acetate 0.015 mol in 25 mL ethanol was refluxed for 6 hrs. The mixture was concentrated and poured in to ice. The precipitate obtained was filtered washed and recrystallised from ethanol.

2.3 *In vitro* Anti-diabetic Screening

2.3.1 Glucose uptake in yeast cells [9]

Yeast cells were prepared by, commercial baker's yeast and was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37℃. Reaction was started by adding 100 µl of yeast suspension, vortex and further incubated at 37℃ for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant. The percentage increase in glucose uptake by yeast cells was calculated using the following formula-

Increase in glucose uptake (%) = Abs sample– Abs control* 100 / Abs sample

Where, Abs control is the absorbance of the control reaction (containing all reagents except the test sample), and Abs sample is the absorbance of the test sample. All the experiments were carried out in triplicates.

2.3.2 Alpha-amylase inhibitory activity [10]

The activity of α-amylase was measured using the starch-iodine method. Briefly, 20 µ l of α-amylase solution (0.030 mg/ml) was mixed with 1.3 ml of Tris-HCl buffer (0.01 M containing 0.006 M NaCl, pH 6.8) and 80 µl of the aqueous extract. After incubation at 37ºC for 20 min, 100 µl of the starch solution (0.1%) was added, and the mixture re-incubated for 20 min, after which

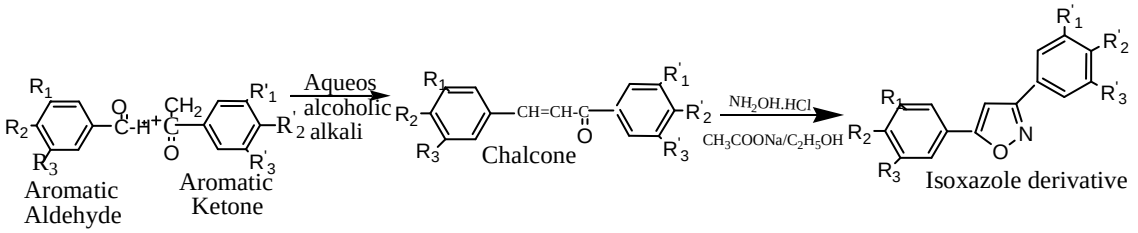

Fig. 1. Scheme of synthesis of the isoxazole derivatives

2 ml of 0.01% acidic iodine solution was added, and the absorbance measured at 565 nm. The percentage inhibition was calculated by comparing to the control which did not have the extract. Inhibition of enzyme activity was calculated as

% = (A-C) X 100 / (B-C)

where, A = absorbance of the sample, B = absorbance of blank (no extract), and C = absorbance of control (no starch).

2.4 Determination of Antibacterial Activity

The antibacterial activity of synthetic products was assessed against three bacteria species: *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM 2079 and *Escherichia coli* NCIM 2931. The respective cultures were prepared in nutrient broth media after incubation at 37℃ for 24 hrs. The antibacterial activity was determined by agar disc diffusion test.

2.5 Agar Disc Diffusion Test [11]

Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube. Using the swab, streak the Nutrient agar plate to form a bacterial lawn. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction. Repeat this rotation 3 times. Allow the plate to dry for approximately 5 minutes.

Use an Antibiotic Disc Dispenser to dispense discs onto the plate and administer the respective samples onto the labelled discs. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar. Plates should be incubated overnight at an incubation temperature of 37℃.

3. RESULTS AND DISCUSSION

None of the tested compounds are superior to Ciprofloxacin which was the standard. Most effective one possesed chlorine in the Phenyl ring attached at 5-C of isoxazole and have NH_2 substitution in phenyl ring attached at 3-C of isoxazole against Gram +ve *Bacillus subtilis*, *staphylococcus aureous* and Gram –ve *E. coli*. A few compounds substituted with nitro/halogen on Phenyl ring at 5-C of isoxazole and ethoxyl group on phenyl ring at 3-C of isoxazole observed to be active only against *E. coli*. Compounds with significant *in vitro* anti-diabetic action by studying the glucose uptake by yeast cell method are with Br/NO_2 substituted forR_2/R_3 in phenyl ring when R'2 is OH/NH_2.

Table 1. IR and NMR spectral details of synthesized compounds

Sample ID	IR peaks(cm^{-1})	NMR peaks (ppm)
L1	1011(N-O-stretch),1500(C-O-stretch),1243 (N-O out of pane bend)	11.663(alcoholic proton) 8-421 (2H),-CH_2-5 membered ring, 8.330, (1H)-isomer of isoxazole, 8.229, 8.2210,8.05,8.04-(4H),of Aro-H.
L2	1104(N-O- stretch),1511(C-O stretch), 1245(N-O out of pane bend)	8.404-(2H),-CH2(5-membered ring).8.357-(1H)isomer of isoxazole,8.363,8.366,8.369-(4H,Ar-H)
L3	1105(N-O stretch),1533(C-O stretch), 1350(N-O out of pane bend)	11.646-alcoholic proton., 8.424-(2H,CH2-5 membered ring),
L4	1018(N-O stretch),1500(C-O	8.337-(2H),CH2(5-membered ring)7.469-(4H) of Ar-H

Sample ID	IR peaks(cm^{-1})	NMR peaks (ppm)
	stretch),1201(N-O out of pane bend)	
L5	1018 (N-O stretch),1500(C-O stretch), 1201(N-O out of pane bend)	7.470 (4H)of Ar-H
L6	1016(N-O stretch),1530(C-O stretch), 1219(N-O out of plane bend)	11.075 – alcoholic proton peak.8.141-(2H)CH2-5-membered ring,7.959,7.955,7.875 (4H,of Ar-H)
L7	1009(N-O stretch),1550(C-O stretch), 1217(N-O out of plane bend)	11.844 alcoholic proton,8.308-(2H)CH2-5 membered ring.7.872,7.850-(4H,Ar-H)
L8	1171(N-O stretch),1517(C-O-stretch), 1245(N-O out of plane bend)	10.966-alcoholic proton,8.306-(2H,CH2-5 membered ring),8.067,7.545,7.539 (4H of Ar-H)
L9	1041(N-O stretch),1491(C-O stretch), 1295(N-O out of plane bend)	11.186-alcoholic proton peak.7.526-(2H,CH2 5-membered ring)
L10	1090(N-O stretch),1490(C-O stretch), 1219(N-O out of plane bend)	8.176-(2H,CH2-5membered ring),8.170-OH isomer of isoxazole.8.157,8.152,8.149-Ar-H
L11	1012(N-O stretch),1490(C-O stretch),1219(C-N stretch),1100(N-O out of plane)	8.175-2H (CH2-of 5-membered ring.)8.157-OH isomer of isoxazole.8.153,7.995-(4H of Ar-H)
L12	1012 (N-O stretch),1218(C-O stretch),1489(C-N stretch),1100(N-O out of plane bend)	8.175- (2H (CH2-of 5-membered ring.)8.157-OH isomer of isoxazole.8.154,7.996-(4H of Ar-H))
L13	1116(N-O stretch),1240(C-O stretch), 1550(C-O carbonyl),1100(N-O out of plane)	8.231- (2H (CH2-of 5-membered ring.)8.227-OH isomer of isoxazole.8.223,7.=8.204-(4H of Ar-H))
L14	1012(N-O stretch),1219(C-N stretch), 1490(C-N stretch)	8.177- (2H (CH2-of 5-membered ring.)8.174-OH isomer of isoxazole.8.153,7.996-(4H of Ar-H))
L15	1040(N-O stretch),1530(C-O-stretch), 1178(C-N stretch)	8.766- (2H (CH2-of 5-membered ring.)8.341-OH isomer of isoxazole.8.321,8.272,8.269-(4H of Ar-H))
L16	1008(N-O stretch),1223(C-N stretch), 1603(C-O stretch)	8.172- (2H (CH2-of 5-membered ring.)8.150-OH isomer of isoxazole.8.004,7.965-(4H of Ar-H))
L17	1016(N-O stretch),1218(C-N stretch), 1527(C-O stretch)	8.793- (2H (CH2-of 5-membered ring.)8.788-OH isomer of isoxazole.8.784,8.362-(4H of Ar-H))
L18	1047(N-O stretch),1260(C-N stretch), 1490(C-O stretch),1179(N-O out of plane)	8.173- (2H (CH2-of 5-membered ring.)8.151-OH isomer of isoxazole.7.990,7.951,7.936-(4H of Ar-H))
L19	1041(N-O stretch),1585(C-O stretch),1183(N-O out of plane)	7.746- (2H (CH2-of 5-membered ring.)7.725-OH isomer of isoxazole.7.707,7.674,7.635-(4H of Ar-H))
L20	1046(N-O stretch),1212(C-N stretch), 1507(C-O stretch),1153(N-O out of plane)	8.749- (2H (CH2-of 5-membered ring.)8.114-OH isomer of isoxazole.8.089,8.070,8.037-(4H of Ar-H))
L21	1041(N-O stretch),1264(C-N stretch), 1519(C-O stretch)	8.294- (2H (CH2-of 5-membered ring.)8.272-OH isomer of isoxazole.8.201,8.177-(4H of Ar-H))
L22	1000(N-O stretch),1507(C-O	8.052- (2H (CH2-of 5-membered ring.) 6.914--(4H of

Sample ID	IR peaks(cm^{-1})	NMR peaks (ppm)
	stretch), 1238(C-N stretch)	Ar-H))
L23	1039(N-O stretch),1129(C-Nstretch), 1492(C-O stretch)	7.503- (2H (CH2-of 5-membered ring.)7.500-OH isomer of isoxazole.7.497,7.494,7.490-(4H of Ar-H))
L24	1051(N-O stretch),1213(C-N stretch), 1507(C-O stretch)	8.320- (2H (CH2-of 5-membered ring.)7.831-OH isomer of isoxazole.7.810,7.691,7.686-(4H of Ar-H)
L25	1060-(N-O stretch),1270(C-N stretch), 1550(C-O stretch)	8.732- (2H (CH2-of 5-membered ring.)8.066-OH isomer of isoxazole.8.032,7.946,7.926-(4H of Ar-H))

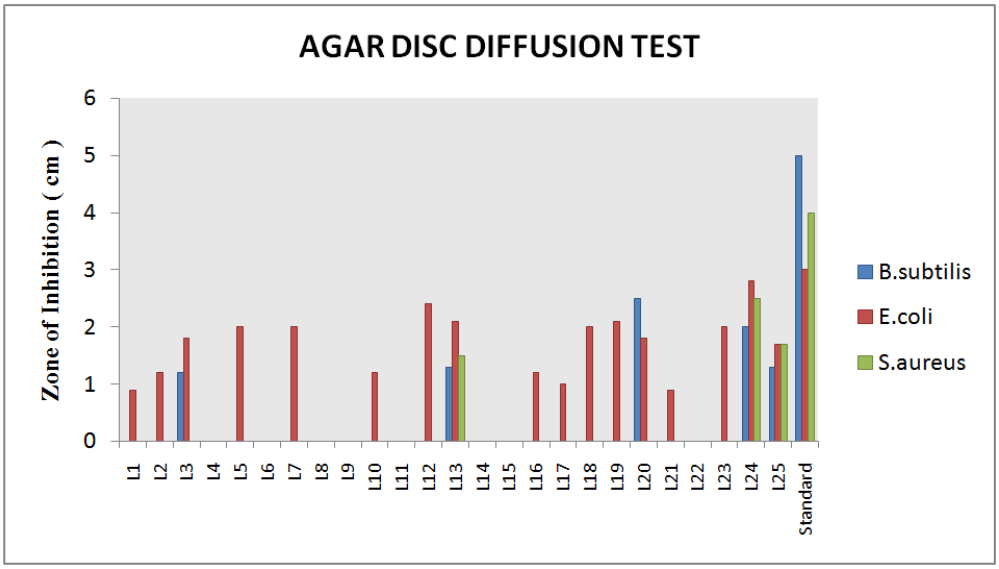

Fig. 2. Anti-bacterial activity of Isoxazole compounds (L1-25) by disc diffusion method using *Bacillus subtilis* NCIM 2063, *Staphylococcus aureus* NCIM 2079 and *Escherichia coli* NCIM 2931

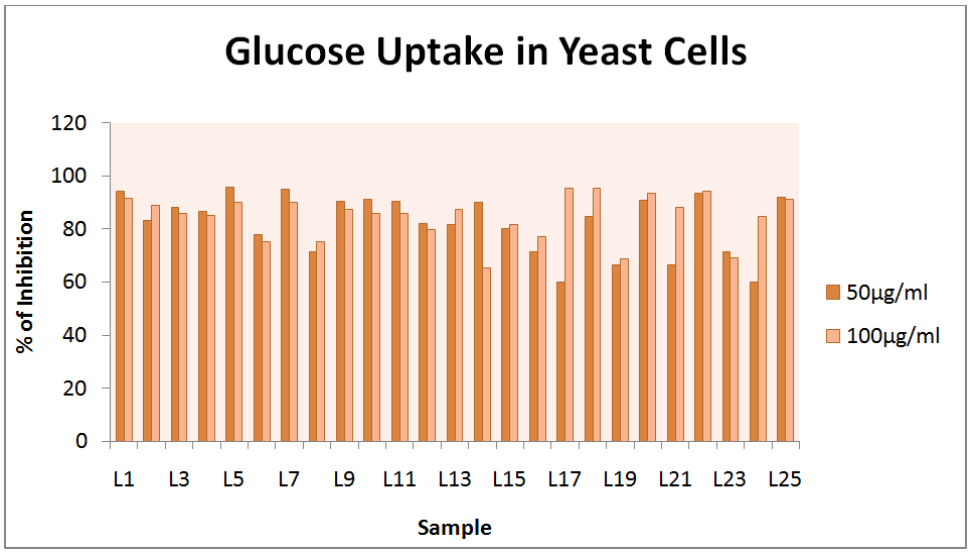

Fig. 3. Anti-diabetic activity of isoxazoles taken in the concentration of 50 µg/ml and 100 µg/ml by studying glucose uptake in living yeast cells

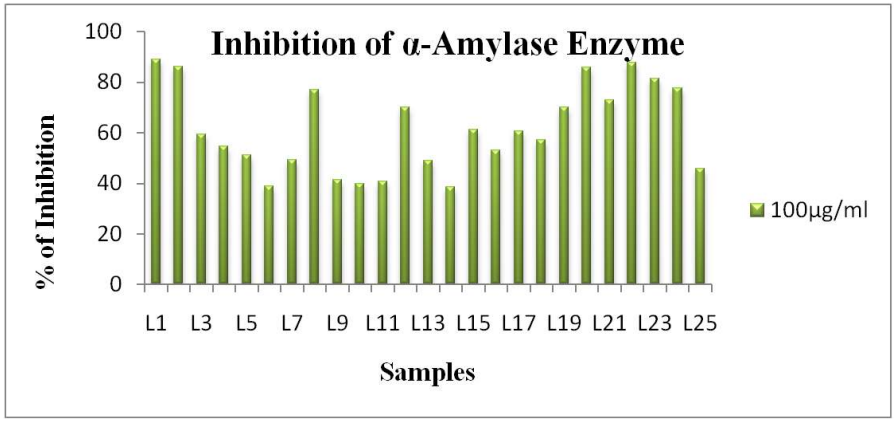

Fig. 4. Anti-diabetic activity of isoxazoles taken in the concentration of 100 µg/ml by inhibition of α amylase enzyme

Table 2. Elemental analysis result of synthesized compounds

	Calculated			Analytical		
Sample	**N%**	**C%**	**H%**	**N%**	**C%**	**H%**
L1	4.43	56.99	3.19	4.65	66.76	3.86
L2	9.92	63.83	3.57	10.59	49.60	3.81
L3	5.16	66.31	3.71	7.67	77.11	8.42
L4	5.27	76.48	5.21	8.45	51.10	3.58
L5	4.43	56.99	3.19	4.65	66.50	3.67
L6	9.92	63.83	3.57	10.64	49.87	2.79
L7	9.92	63.83	3.57	5.20	67.00	4.02
L8	4.71	68.68	5.09	4.65	66.98	4.30
L9	4.71	68.68	3.85	5.61	67.46	5.80
L10	4.58	58.85	2.96	3.80	54.30	3.60
L11	4.46	61.17	3.85	4.62	66.74	4.90
L12	9.99	68.56	4.32	10.62	49.81	3.02
L13	5.19	71.25	4.48	8.48	50,91	3.77
L14	5.62	81.90	6.06	5.19	67.85	3.75
L15	4.46	61.17	3.85	4.60	67.39	5.47
L16	9.99	68.56	4.32	10.64	68.50	6.02
L17	9.99	68.56	4.32	10.60	49.68	4.27
L18	4.07	59.32	4.10	4.10	69.71	5.16
L19	9.03	65.80	4.55	5.64	78.69	6.90
L20	4.32	61.83	4.51	3.24	59.18	4.44
L21	8.89	57.16	3.52	6.31	46.17	2.60
L22	4.89	55.71	6.08	5.96	50.98	7.98
L23	10.35	66.55	4.10	11.03	70.76	6.03
L24	10.35	66.55	4.10	10.69	68.89	6.38
L25	11.19	76.78	5.64	10.67	68.74	6.42

4. CONCLUSION

Presence of halogenated aromatic ring at 5-C and amine substituted phenyl ring at 3-Cof isoxazole exhibited moderate anti-bacterial activity. In the anti-diabetic study halogenated or nitrated phenyl ring at 5-C and hydroxyl/amine substituted phenyl ring at 3-C of isoxazole exhibited anti-diabetic action.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as

specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

DISCLAIMER

Conference name: "Drug Discovery Conference" Link:"http://www.innovabalt.eu/pictures/zinas/225.pdf" (August 27-29, 2015, Riga, Latvia).

ACKNOWLEDGEMENT

The authors are acknowledge to DST-SERB – India for providing the fund and expressing thanks to STIC of Cochin University for spectral Analysis.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jung HK, Doddareddya MR, Cha JH, Rhim H, Cho YS, Koh HY, et al. Synthesis and biological evaluation of novel T-type Ca 2+ channel blockers. Bioorg Med Chem. 2004;12:3965–70. (PubMed)
2. Popat KH, Nirmavat KS, Kachhadia VV, Joshi HS. Synthesis and biological activity of 3-aryl-5-(3'- bromo/chlorophenyl) isoxazoles. J Indian Chem Soc. 2003;80: 707–8.
3. Norman BH, Lander PA, Gruber JM, Kroin JS. Cyclohexyl-linked tricyclic isoxazoles are potent and selective modulators of the multidrug resistance protein (MRP1) Bioorg Med Chem Lett. 2005;15:5526–30. (PubMed)
4. Calí P, Naerum L, Mukhija S, Hjelmencrantz A. Isoxazole-3-hydroxamic acid derivatives as peptide deformylase. Bioorg. Med. Chem. Letters. 2004;14(24): 5997-6000.
5. Shantaram Khanage, Popat Mohite, Ramdas Pandhare, Appala Raju, Synthesis and pharmacological evaluation of isoxazole derivatives containing 1,2,4-triazole Moiety. Marmara Phrmaceutical Journal. 2012;16:134-140.
6. Ravi SL, Nitinkumar SS, Ravindra RK, Imtiyaz MK La-mani RS, Shetty NS, Kamble, RR, Khazi IA. Synthesis and antimicrobial studies of novel methylene bridged ben- isoxazolyl imidazo[2,1-b][1,3,4]thiadiazole derivatives. EurJ Med Chem. 2009;44:2828-33.
7. Kumar A, Maurya RA, Sharma S, Ahmad P, Singh AB, Tamrakar AK, Srivastava AK. Design and synthesis of 3,5-diarylisoxazole derivatives as novel class of anti-hyperglycemic and lipid lowering agents. Bioorg Med Chem. 2009; 17:5285-92.
8. Zhou Jie-Wen, Yan Ju-Fang, Tang Xue-Mei. Synthesis and preliminary evaluation of antidiabetic activity for β-amino ketone containing isoxazole moiety. Chin. J. Org. Chem. 2010;30(04):582-589.
9. Daksha G, Chandrashekar Lobo, Yogendra R, Nilesh G. *In-vitro* antidiabetic activity of stem bark of *Bauhinia purpurea* Linn. Der Pharmacia Lettre. 2012;4(2):614-619.
10. Etoundi CB, Kuate D, Ngondi JL, Oben J, Anti- amylase, anti lipase and anti oxidant effects of aqueous extracts of some Cameroonian spices. Journal of Natural Products. 2010;3:165-171.
11. Coyle MB. Manual of antimicrobial susceptibility testing: Test methods. America Society of Microbiology. 2005;39-46.

© 2016 Joseph and George; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/12300