IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 12, Issue 4 Ver. VI (Jul – Aug 2017), PP 14-20
www.iosrjournals.org

Biochemical and Pharmacological Studies of the Condensed Products of α, β Unsaturated Ketones: Docking Studies

*Manish Rapolu[1], M. Srinivasamurthy[2]
[1]*Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Deshmukhi, Hyderabad, India.*
[2]*Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Deshmukhi, Hyderabad, India.*
*Corresponding Author: *Manish Rapolu*

Abstract: *α, β-Unsaturated ketones commonly known as chalcones are an important class of organic compounds being studied over the years and reported to possess wide spectrum of biological properties such as antibacterial, antifungal, antitubercular, antimalarial, anti-inflammatory, antileishmanial ,anticancer and antioxidant activities. The presence of enone function in the chalcone molecule confers the biological activity, the importance of which is well documented in the literature. In the present work three new series of α, β-unsaturated ketones have been synthesized by reacting Acetophenone with 4-carboxyl benzaldehydes by Claisen-Schmidt condensation followed by reaction with amino triazole. The compounds have been characterized by UV, IR, 1H NMR, Mass spectral data and elemental analysis. All the synthesized compounds have been evaluated for their in vitro antibacterial and antioxidant activities. Most of the compounds exhibited antibacterial property at a concentration of 100 µg/ml. All the compounds exhibited antioxidant property with EC50above 500 µg/ml.*

Keywords: *α, β-Unsaturated ketones, Antioxidant activity*

Date of Submission: 05-07-2017 Date of acceptance: 05-08-2017

I. Introduction

The search for 'better medicines for a better world' is a never-ending process to help the suffering mankind from dreadful and fatal ailments. The process of new drug discovery is driven by the requirement to synthesize novel molecules having good potential with high therapeutic index[1].

α, β-Unsaturated ketones also known as chalcones have recently attracted the attention of many medicinal chemists owing to the ease of their synthesis and wide array of pharmaceutical and medicinal applications. Chalcones are abundant in the plant kingdom[2]. They are considered to be the precursors of flavanoids and isoflavanoids. Chemically they consist of two aromatic rings joined by a three carbon α, β-unsaturated carbonyl system. Synthesis of chalcones is generally accomplished by a simple base catalysed Claisen-Schmidt condensation of a ketone and a suitably substituted aldehyde. It is now well known that most natural and synthetic chalcones have shown extensive pharmacological activities such as antiprotozoal, antifungal, anti-inflammatory, antileishmanial, nitric oxide inhibition, inhibition of the production of the interleukin-1, anticancer, antibacterial and antioxidant[3]. Keeping in view these diverse therapeutic activities, it was contemplated to synthesize a novel series of chalcones. In the present work attention has been focused on the synthesis of chalcones with different ketone moieties and their antibacterial and antioxidant properties.

Materials And Methods

Chemistry: All chemical were purchased from commercial sources. The melting points of all the compounds weredetermined by open capillary and are uncorrected/ unchanged. The purity test was done by TLC method. IR spectra were recorded in KBr on Shimadzu FT-IR 8300 spectrophotometer[1].H NMR spectra were recorded on Varian 400 MHz spectrometer using DMSO as solvent and tetra methyl silane as an internal standard. Mass spectra were recorded on Aglient 6430 Triple Quadruple LC-MS system.

1.1 Synthesis of chalcones [I- III][9]: Quantities of 4-carboxyl benzaldehyde (0.01mol) and acetophenone(0.01 mol) were dissolved in minimum amount of alcohol. Sodium hydroxide solution (0.02 mol) was addedslowly and the mixture stirred for 2hrs until the entire mixture becomes very cloud. Then the mixture was poured slowly into 400 ml of water with constant stirring and kept in refrigerator for 24 hours. The precipitate obtained was filtered, washed and recrystallized from ethanol. Finally, the compounds synthesized were, 3-(4- methoxyphenyl)-1phenylprop-2-en-1-one (I), 3-(3-chlorophenyl)-1-phenylprop-2-en-1-

one (II), and 3(3-nitrophenyl)-1-phenylprop-2-en-1-one (III) respectively. The completion of the reaction was monitored by TLC.

1.2 Preparation of hydrazide derivatives of chalcone[I- IIIa, I-IIIb][10]: Appropiate quantities of acid(0.1mole) ethanol (50ml) was introduced into a clean and dry round-bottemed flask and stirred well for 10min.To the above mixture,few drops of concentrated sulphurric acid was added and the reaction mixture was concentrared by distilling the excess ethanol under reduced pressure and treated with saturated solution of sodium -bi-carbonate.The ester formed in the reaction was used for the preparation of hydrazide directly.The appropriate aster (0.1 mole) was dissolved in 50 ml of ethanol in a clean dry round -bottmed flask and to this hydrazine hydrate (0.1mole) was added.The reaction mixtutre was then refluxed for a period of 15 to 18 hrs.The excess ethanol was distilled off under reduced pressure.The resultant mixture was then poured into ice cold water and the obtained solid was filtered,recrystallized from ehanol.

1.3 Preparation of ester derivatives of furan derivatives [I-IIIa,I-IIIb][10]: Appropiate quantities of furan 2-carboxylic acid (0.1mole) ethanol (50ml) was introduced into a clean and dry round-bottemed flask and stirred well for 10min.To the above mixture,few drops of concentrated sulphurric acid was added and the reaction mixture was concentrared by distilling the excess ethanol under reduced pressure and treated with saturated solution of sodium -bi-carbonate.The ester formed in the reaction was used for the preparation of amide directly..

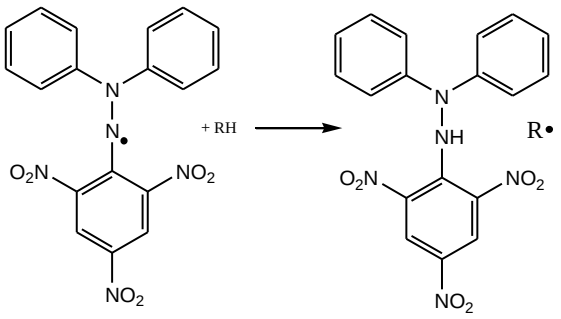
1.4 Preparation of amide derivatives of chalcone Derivatives [11]: The appropriate aster form of furan (0.1 mole) was dissolved in 50 ml of ethanol in a clean dry round -bottmed flask and to this amino triazole (0.1mole) was added.The reaction mixtutre was then. refluxed for a period of 18 to 20 hrs.The excess ethanol was distilled off under reduced pressure.The resultant mixture was then poured into ice cold water and the obtained solid was filtered,recrystallized from ehanol.

1.5 (E)-N-(4-(3-(3,4,5-Trimethoxyphenyl)-3-oxoprop-1-en-1-yl)benzoyl)-Furan-2-carbohydrazide solvent system (CF-6):TLC solventsystem:n-Hexane:Ethyl acetate (3:2), Rf value: 0.76. IR (KBr, cm-1): 1635(NH-CO-),2349 (C-O-C str), 1487 (Ar C=C), 1355 (Ar-C-O-C), 1590 (C=N), 3070 (N-H). ^{1}H-NMR (DMSO-d6400 MHz, δ ppm): 7.98(s,2H of Ar-H),7.94(d,2H of Ar-H),7.56(d,2H of Ar-H),7.52(d,1H of Furan),6.82(t,1H of Furan),8.08(d,1H of Furan ring),8.06(d,1H ofCH=CH-C=O), 7.22(d,1H of CH=CH-C=O),8.1(s,2H of - NH),3.81(s,9H ofOCH3)). ^{13}C NMR:
56.1,56.156.1,107.1,112.3,115.3,111.7,121.3,122.5,123.2, 127.4,127.4,
129.2,129.2,131.2,136.6,145.8,1451,147.0,150.3,156.3,157.1,164.8,196.4 . Mass Spectrophotometry (m/z): 452.13 (M+1).

1.6 (E)-N-(4-(3-(3,4,-Dimethoxyphenyl)-3-oxoprop-1-en-1-yl))benzoyl)-Furan-2-carbohydrazide solvent system (CF-7): solventsystem:n-Hexane:Ethyl acetate (3:2), Rf value: 0.76. IR (KBr, cm-1): 16352349 (C-O-C str), 1487 (Ar C=C), 1355 (Ar-C-O-C), , 1590 (C=N), 3070 (N-H). ^{1}H-NMR (DMSO-d6400 MHz, δ ppm): 7.98(s,2H of Ar-H),7.94(d,2H of Ar-H),7.56(d,2H of Ar-H),7.52(d,1H of Furan),6.82(t,1H of Furan),8.08(d,1H of Furan ring),8.06(d,1H ofCH=CH-C=O), 7.22(d,1H of CH=CH-C=O),8.1(s,2H of -NH), 3.81(s,6H of OCH3)^{13}C NMR:
56.1,56.1,107.1,112.3,115.3,111.7,121.3,122.5,123.2,127.4,127.4,129.2,129.2,131.2,136.6,145.8,145.1,147.0,150.3,156.3,157.1,164.8,196.4. Mass Spectrophotometry (m/z): 421.13 (M+1).

1.7 Pharmacological activity:**Method followed:** DPPH method[25, 86]

The DPPH Method: A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown:



(DPPH free radical-purple) (Reduced DPPH-H-Yellow)

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured.

Working Procedure: DPPH˙ solution
A working solution of DPPH˙ having an absorbance of 0.9 at 516 nm was used . this was prepared by taking 95 µL of stock solution containing 12.9 mg of DPPH˙ in 10 mL of methanol.

Standard solution: Ascorbic acid was used as a standard free radical scavenger. This was prepared by dissolving 50 mg of ascorbic acid in 50 mL of methanol.

Test solution: Test solutions of the compounds (10mg/10mL) were prepared by dissolving them in 1 mL DMSO and volume was made to 10 mL with methanol.

Procedure: To 95 µL DPPH˙ solution in methanol, different concentrations of ascorbic acid wereadded and the volumes were made upto 4 mL with methanol. DPPH˙ diluted to 4 mL was taken as blank. Decrease of absorbance in the presence of ascorbic acid was noted down after 15 minutes. Linear regression was applied for concentration and percentage inhibition and EC_{50} was calculated. To the different concentrations of test solutions (0.4,0.8, 1.2, 1.6 mL), 95 µL of DPPH˙ solution was added and volume made upto 4 mL with methanol. Decrease in absorbance of DPPH˙ was noted after 15 minutes. Linear regression was applied for concentration and percentage inhibition and EC_{50} was calculated from graph.

1.8 Molecular docking: Molecular docking studies by using GLIDE XP module of Schrodinger suite were performed for the selected quinazoline derivatives which were screened for in-vitro Antioxidant activity. Initially, a digitalized structure of the protein antooxidant was retrieved from the protein data bank with pdb id 1cb4 (COPPER, ZINC SUPEROXIDE DISMUTASE) Structure of the protein was processed by adding hydrogen to satisfy the valence and optimized by using OPLS-2005 force field (optimized potential for liquid simulations). Receptor grid generation was accomplished using Glide docking protocol and ligands were docked by employing XP mode of Glide. Best pose of each ligand was ranked according to the E-model energy. The docking score from Glide (Glide Score) is entirely based on Chem Score. It also include a steric – clash term, adds polar terms featured by Schrodinger to correct electrostatic mismatches.G score = 0.065 x Van der Waals energy + 0.130 x Coulomb energy + Lipophilic tern (Hydrophobic interaction) +H bonding + Metal binding + Bury P (Penalty for buried polar groups) + Rot B (Penalty for freezing rotatable bond) +Site (Polar interactions in the active site) [16].

II. Results And Discussion

In the present, Chalcones (3a-3g) were prepared by base catalysed claisen-schmidit condensation between 4-formyl Benzaldehyde and different acetophenone further, compounds 6a-6af were treated with hydrazine hydrate to give series of chalcone derivatives (4a-4f) followed by interaction of these compounds 4(a-g) with ester form of furan in the presence of acetic acid to give a series of furan linked chalcone derivatives 8(a-g). Compound 3 was confirmed by NMR, C13 NMR and Mass spectroscopy.The final compounds chalcone linked furan derivatives 8(a-f) were confirmed by (-NH-CO) -amide peak appear around at δ 8.0 in proton NMR as singlet and protons peaks of-CH=CH-C=O appeared at 8.06 and 7.59 respectively .The thiazine linked furan were confirmed by C13 NMR and mass spectral data.

Table 1: Physical Characterization data of chalcone linked furan derivatives:

Product Code	R	X	Molecular Formula	Molecular weight	Solvent for recrystallization	M.P (^{0}C)	Yield (%)
CF1	2-Cl	C_6H_5	$C_{21}H_{15}ClN_2O_4$	394.07	Ethanol	149	56
CF2	4-OH	C_6H_5	$C_{21}H_{16}N_2O_5$	376.11	Ethanol	106	62
CF3	3-NO_2	C_6H_5	$C_{21}H_{16}N_3O_6$	405.1	Ethanol	121	63
CF4	H	C_6H_5	$C_{21}H_{16}N_2O_4$	360.11	Ethanol	130	71
CF5	4-CH_3	C_6H_5	$C_{22}H_{18}N_2O_4$	374.13	Ethanol	112	55
CF6	$(OCH_3)_3$	C_6H_5	$C_{24}H_{22}N_2O_7$	450.44	Ethanol	142	61
CF7	$(OCH_3)_2$		$C_{23}H_{20}N_2O_6$	420.41	Ethanol	135	70

Table 2: Data Showing Anti-Oxidant Activity of Chalcone Linked Furan **[CF_{1-7}]**

Compound No.	Antioxidant activity (EC50 in µg/mL)
CF-1	579
CF-2	596
CF-3	577
CF-4	574
CF-5	540
CF-6	605
CF-7	588

Molecular docking:

Molecular docking study was performed for further exploration of the mechanism of action of thesynthesized compounds with anti-oxidant enzyme and to elucidate the observed biological results. Docking of of compound compound CF-1 showed 4 hydrogen bond interaction with ARG 141,HIE 61 and ASN 63. In addition to this, compound CF-1 has trimethoxy group which provided additional interaction with active site amino acid receptor and this might be contributed to better activity than remaining copmpounds. Further, this is supported by results obtained from antioxidant activity.

.

The two dimentional and three dimentional represent of compound CF-1-a were given below.

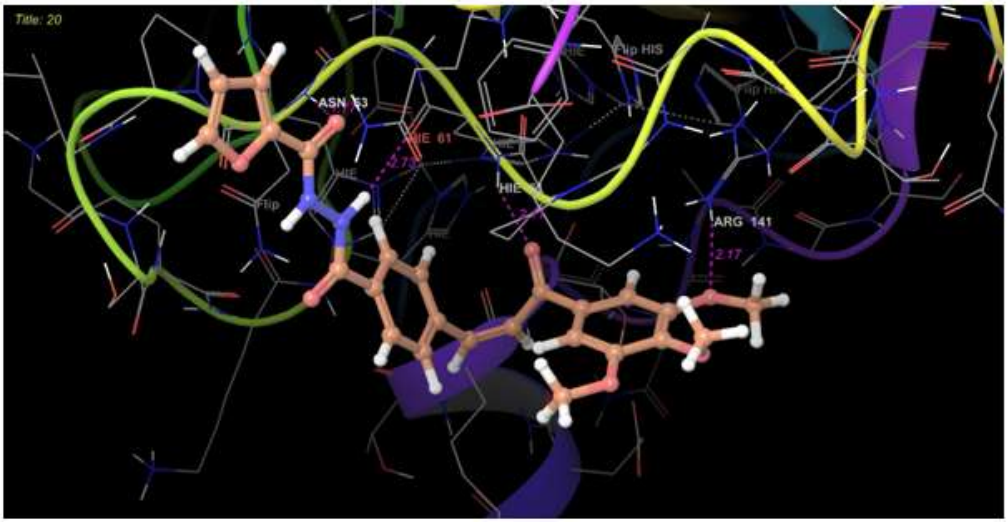


Fig 1: Three – dimensional structural model of compound CF-6 into anti oxidant enzyme

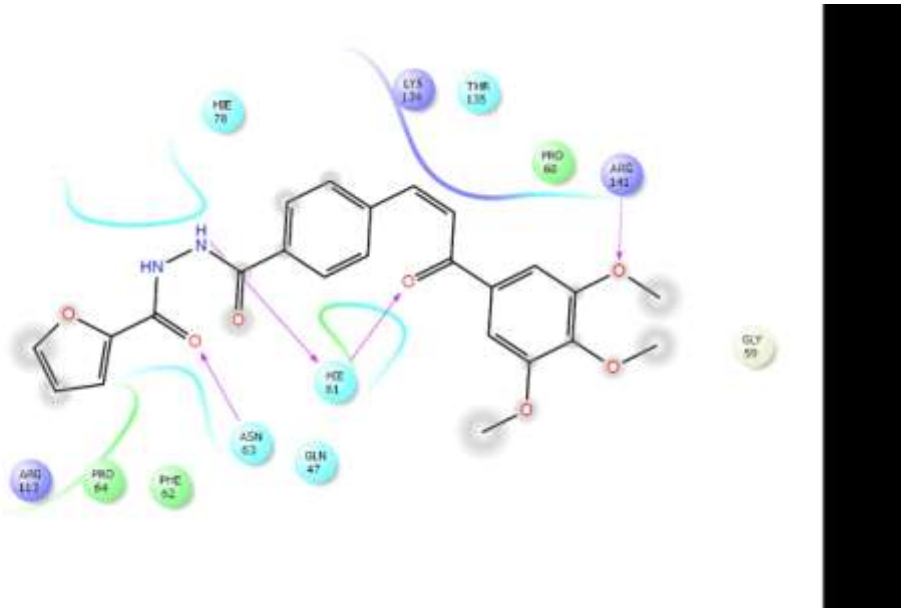


Fig 2: Two – dimensional representation of the interacting mode of CF-6 with anti oxidant receptor

Docking Score:1cb4 dock

Compound	Dock score	No of H-bonds	Interacting amino acids	H-bond distance	Bond energy
CF-6	-4.842	4	ASN 63 HIS 61 ARG 141	1.77 2.73, 2.11 2.17	-46.061
CF-2	-4.364	2	HIS 61 HIS 78	2.13 2.22	-43.31
CF-7	-3.644	3	ASN 63 HIS 63 ARG 141	2.02 2.47 1.96	-40.337
CF-1	-2.769	1	HIS 61	2.12	-29.436
CF-5	-3.487	0	-	-	-42.578
CF-3	-3.431	2	ASN 63 ARG 141	2.16 2.15	-44.444

III. Conclusion

The synthesized compounds were evaluated for in-vivoanti-oxidant activity. Among the evaluated compounds CF-7-hexhibited highest inherent anti-oxidant activity due to electron donating character of trimethoxy group on phenyl nucleus. In addition to this, CF-2 compound showed significant docking interaction with anti oxidant receptor active site. Based on these observation, ATA-a have proven the potential as valuable lead for anti-oxidant activity and remaining compounds exhibited mild to moderate activity compared to the standard compound(Ascorbic acid).

Acknowledgment

Dr.M.Srinivasa Murthy, Principal, Vignan Institute of Pharmaceutical Sciences for providing facilities to carry out the research work and also thankful to G. Deepak Reddy for providing docking study.

References

[1]. Chiaradia LD, Santos RD, Vitor CE, Vieira AA, Leal PS, Nunes RJ et al. Synthesis and pharmacological activity of chalcones derived from 2,4,6-trimethoxyacetophenone in RAW 264.7 cells stimulated by LPS: Quantitative structure-activity relation ships. Bioorg Med.Chem. 2008.
[2]. Shukla P, Singh AB, Srivastava AK, Pratap R. Chalcone based aryloxypropanolamines as potential antihyperglycemic agents. Bioorg Med Chem Lett. 2007; 17:799-802.
[3]. Liu XL, Xu YJ, Go ML. Functionalised chalcones with basic functionalities having antibacterial activity against drug sensitive Staphylococcus aureus. Eur J Med Chem. 2007; 1-7..
[4]. Selvakumar N, Kumar GS, Azhagan AM, Rajulu SS, Kumar MS, Das J, et al. Synthesis, SAR and antibacterial studies on novel chalcone oxazolidinone hybrids. Eur J Med Chem. 2007; 42:538-43.
[5]. Batovska D, Parushev S, Slavova A, Bankova V, Tsvetkova I, Ninova M, et al. Study on the substituents' effects on a series of synthetic chalcones against the yeast Candida albicans. Eur J Med Chem. 2007; 42:87-92.
[6]. Nowakowska Z, Kedzia B, Schroeder G. Synthesis, physiochemical properties and antimicrobial evaluation of new (E)-chalcones. Eur J Med Chem. 2007; 1-7.
[7]. Yar MS, Siddiqui AA, Ali MA. Synthesis and evaluation of phenoxy acetic acid derivatives as a anti-mycobacterial agents. Bioorg Med Chem Lett. 2006; 16:4571-4.
[8]. 2,5-dimethylthiophene/furan with aromatic aldehydes in water: Synthesis of (2*E*)-3-aryl-1-
[9]. (thien-3-yl)-prop-2-en-1-ones. Ind J Chem. 2006; 45B:1936-41.
[10]. Halnor VB, Dalvi NR, Joshi NS, Gill CH, Karale BK. Condensation reactions of various nucleophiles with 3-formylchromone. Ind J Chem. 2006; 45B:288-91
[11]. Nishida J, Kuwabata J. DPPH radical scavenging reaction of hydroxyl- and methoxychalcones. Biosci Biotechnol Biochem. 2006; 70(1):193-202.
[12]. Lawrence NJ, Patterson RP, Ooi LL, Cook D, Ducki S. Effects of α-substituents on structure and biological activity of anticancer chalcones. Bioorg Med Chem Lett. 2006; 16:5844-8.
[13]. Boeck P, Leal PC, Yunes RA, Filho VC, Lopez S, Sortino M, et al. Antifungal activity and studies on mode of action of novel xanthoxyline-derived chalcones. Arch Pharm Chem Life Sci. 2005; 338:87-95.
[14]. Dominguez JN, Leon C, Rodrigues J, Dominguez NGD, Gut J, Rosenthal PJ. Synthesis and antimalarial activity of sulfonamide chalcone derivatives. Farmaco. 2005; 60:307-11.
[15]. Alvarez MDLA, Zarelli VEP, Pappano NB, Debattista NB. Bacteriostatic action of synthetic polyhydroxylated chalcones against *Eschechria coli*. Biocell. 2004; 28(1):31-4.
[16]. Xue CX, Cui SY, Hu ZD, Fan BT. 3D QSAR studies on antimalarial alkoxylated and hydroxylated chalcones by COMFA and COMSIA. Eur J Med Chem. 2004; 39:745-53.
[17]. Lunardi F, Guzela M, Rodrigues AT, Correa R, Mangrich IE, Steindel M, et al. Trypanocidal and leishmanicidal properties of substitution containing chalcones. Antimicro Agents and Chemother. 2003; 47.4:1449-51.
[18]. Tripathi KD. Essentials of Medical Pharmacology. 5th ed. Jaypee Brothers Medical Publishers (P) Ltd, New Dehli. 2003.
[19]. Shetgiri PP, D'Mello PM. Indian Drugs. 2003; 40(10):1-3.
[20]. Rojas J, Paya M, Dominguez JN, Ferrandiz ML. The synthesis and effect of fluorinated chalcone derivatives on nitric oxide production. Bioorg Med Chem Lett. 2002; 12:1951-4.
[21]. Dominguez JN, Charris JE, Lobo G, Dominguez NGD, Moreno MM, Riggione F, et al. Synthesis of quinolinyl chalcones and evaluation of their antimalarial activity. Eur J Med Chem. 2001; 26:555-60.
[22]. Kar A. Medicinal Chemistry. 2nd ed. New Age International Publishers, New Delhi; 2001.
[23]. Arty IS, Timmerman H, Samhoedi M, Sastrohamidjojo, Sugiyanto, Goot HVD. Synthesis of benzylidieneacetophenones and their inhibition of lipid peroxidation. Eur J Med Chem. 2000; 35:449-57.

[24]. Black JG. Microbiology-Principles and exploration. 4th ed. New Delhi: Prentice Hall Publishers; 1991.
[25]. JG. Microbiology-Principles and exploration. 4th ed. New Delhi: Prentice Hall Publishers; 1991.
[26]. Dyer JR. Application of absorption spectroscopy of organic compounds. 1st ed. New Delhi; Prentice Hall Publishers: 1991.
[27]. Silverstein RM, Bassler GC, Morill TC. Spectrometric identification of organic compounds, 5th ed. New York; John Wiley and son Publishers; 1991.
[28]. Foye WO. Principles of Medicinal Chemistry. 3rd ed. Verghese Publishing House, Bombay.1989.
[29]. Harborne JB. The flavonoids, Advances in Research. Chapman and Hall. New York NY, 1988 :329-88.
[30]. Vogel AI, Practical Organic Chemistry. 4th ed. Bungay: ELBS and Longman Publishers; 1978.
[31]. www.en.wikipedia.org
[32]. www.americanchronicle.com
[33]. www.herbal-powers.com

IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS) is UGC approved Journal with Sl. No. 5012, Journal no. 49063.