Synthesis, Characterization and Study of Biological Activity of Novel 4- Hydroxy Azo Coumarine Derivatives.

Mr. S.S. Anjanikar[1], Dr. S.S. Chandole[*2]

[1] Asst. professor and Head, Department of Chemistry, Sharadchandra College, Naigaon.

[*2]S.S. Chandole, Associate Professor, Department of Chemistry, S.G.B. College, Purna Jn.

schandole@reddifmail.com

ABSTRACT

3-(2-(pyrimidin-2-yl)diazenyl)-4-hydroxy-2H-chromen-2-one, (3Z)-3-(2-(pyrimidin-4-yl)diazenyl) -4-hydroxy-2H-chromen-2-one, (3Z)-3-(2-(2H-1,2,4-triazol-3-yl)diazenyl)-4-hydroxy-2H-chromen--one and (3Z)-3-(2-(1,5-dihydro-1,2,4-triazol-4-yl)diazenyl)-4-hydroxy-2H-chromen-2-one were synthesized by coupling of 2-amino pyrimidine, 4-amino pyrimidine, 2H-1,2,4-triazol-3-amine and 2H-1,2,4-triazol-4-amine with 4-hydroxy-2H-chromen-2-one. These 4-Hydroxy Azo Coumarine derivatives were characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. In vitro biological screening effects of the synthesized compounds were tested for their antibacterial and antifungal activity. For antibacterial activity the bacterial species used were *Bacillus subtilis, Escherichia coli, Salmonella typhi, and Staphylococcus aureus* by Agar cup method while fungal species used *Aspergillus flavus, Penicillium chrysogenum, Aspergillus niger and Fusarium moneliforme* by the poison plate method.

Key word: 4-hydroxychromen-2-one, amino pyrimidine, amino1,2,4,triazole, Spectra, biological activity.

INTRODUCTION

Today number of dyes are available in market, in which azo compounds plays a important role which contains at least one N=N usually attached to aromatic compounds. Azo compounds do not occur naturally but synthesized only through chemical synthesis. These compounds were generally synthesized by diazotization of [illegible] amines and later coupled with nucleophiles.

Co-ordinator IQAC Shri Guru Buddhiswami Mahavidyalaya Purna (Jn) Dist. Parbhani - 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Samanta's Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Estd.1983

PRINCIPAL Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Dist.Parbhani

They absorb visible light and appear to be colored hence most important class of synthetic coloring materials.[1]

Azo dyes shows application in photosensitivity, photodynamic therapy, and photographic system as well as in organic photo conductive material.[2] Coumarine is not self fluorescent but when electron withdrawing group such as diazo group attached to it then becomes fluorescent.[3] These azo coumarine derivatives show wide application in biological activities such as anti-convuslant,[4] anti-bacterial,[5] anti-fungal,[6] Anti inflammatory,[7] antiallergic,[8] antioxidant.[9] In many research it is observed that the activity of azo compound increases on the incorporation of suitable heterocyclic moiety. 1,2,4-triazol and pyrimidine are class of heterocycles having significant application in biological activity including antimicrobial, anticancer, antioxidant, antibiotic.[10-11]

In the views of above facts, we are reporting the novel aryl azo 4-hydroxy coumarine compounds prepared and characterized by IR, ^{1}HNMR, ^{13}CNMR and mass spectral analysis. In vitro biological screening effect of the synthesized compounds was tested against the bacterial species using Agar cup method while Fungal were tested by the poison plate method.

EXPERIMENTAL SECTION

The solvents and the reagents used in present study were of analytical grade and obtained from E-Merck and S. D. fine Ltd. Melting points were determined in an open capillary tube and are uncorrected. The purity of the compound has been checked by TLC. The C, H, N analysis of synthesized compounds were carried out by micro combustion method using CHNSO, EA1108, Elemental analyzer model-CARLO-ERBA Instruments, at micro analysis division, National Chemical Laboratory, Pune. The samples weighing between 1-10 mg were used for the analysis. The molecular stoichiometry of each compound was established on the basis of elemental analysis. IR spectra were recorded in $CHCl_3$ on a Shimadzu FTIR-8300 spectrophotometer. The ^{1}H NMR (300 MHz) and ^{13}C NMR (70 MHz) were run on a Bruker Avance DPX-250 spectrometer in CDCl3 using tetramethylsilane as an internal standard. Chemical shift values are given in δ scale. Mass spectra were recorded on Finnigan Mat LCQ Mass Spectrometer using methanol as mobile phase.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

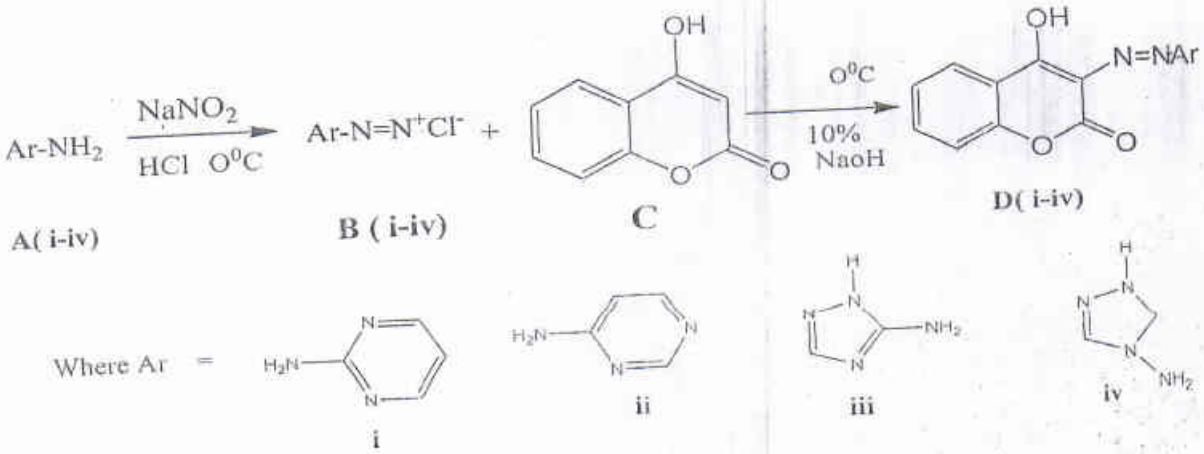
PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

The in vitro biological screenings of the investigated compounds were tested against the bacterial species by agar cup method and fungal species by the poison plate method.

Procedure:

Amino substituted hetero aryl compounds **(A i-iv)** (5mmol) were dissolved in 8ml water and 5ml conc. HCl, mixture is heated until amine hydrochloride is completely dissolved. $NaNO_2$ (5mmol) solution was prepared by dissolving it in minimum quantity of water and kept both the reaction mixture in ice bath for cooling. When these mixtures attain 0-5^{0}C temperature then $NaNO_2$ solution was added to the Amino substituted heteroaryl solution drop-wise with vigorous stirring. Near 0^{0}C temperature was maintained throughout the reaction. After the complete addition reaction mixture was kept in ice bath for 15 minutes with occasional stirring.

The diazotized reaction mixture **(B i-iv)** was then poured in ice cooled solution of 4-hydroxy coumarine **(C)** (5mmol) in 25 ml of 10% sodium hydroxide solution. This mixture was allowed to stand (0-5^{0}C) for 2 hours and then filtered. The crude product thus obtained were dried and recrystallized from acetic acid to give the corresponding compounds **(D i-iv)**.



Reaction Scheme

Antibacterial Activity

The antibacterial activity was measured by agar cup method[9]. The bacterial strains used as test organism were Escherichia coli and Staphylococcus aureus and Salmonella typhi as a gram negative

Co-ordinator IQAC Shri Guru Buddhiswami Mahavidyalaya Purna (Jn) Dist. Parbhani

PRINCIPAL Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Dist.Parbhani

bacterial strains and Bacillus subtils as gram positive bacterial strains. Nutrient agar (Himedia) was prepared and sterilized and kept for 15 minutes in the autoclave. All bacteria were cultured aerobically at 37°C in LB agar and LB broth medium. Before experimental use, cultures from agar medium were sub cultivated in liquid media, incubated for 12 h (37°C). Cups of 10mm diameter were made in the agar plate with sterile cork borer. 100 µl of compound solution prepared in DMF (0.1%) was added in the cups under aseptic condition with the help of micropipette. 100µl of DMF was placed in separate cups as blank (negative control). 100 µl of solution of penicillin in DMF (0.1%) was also placed on the seeded nutrient agar surface as standard reference antibiotic (positive control). The plates were allowed for diffusion of the compound from agar cup into the medium. Then the plates were incubated for 24 hours. Record the zone of inhibition of bacterial growth around the agar cup in millimeter (mm) using zone reader.

ANTIFUNGAL ACTIVITY

Antifungal activity was performed by Poison plate method[10]. A culture of Potato Dextrose Agar (PDA) medium for test of fungi was used. The compound to be tested is added to the sterile medium in aseptic condition. A plate with DMF was prepared as blank (negative control) similarly a plate with 1% Gresiofulvin was prepared as standard reference plate (positive control). For testing the fungal activity *Aspergillusniger, Penicilliumchrysogenum, Fusariummoneliforme, Aspergillusflavus* were selected. They were allowed to grow on slant for 48 hours so as to get profuse sporulation. 5ml of 1:100 aqueous solution of Tween 80 was added to the slant and spores were scraped with the help of Nichrome wire loop to form suspension. The plates were incubated at room temperature for 48 hours. After incubation plates were observed for the growth of inoculated fungi. Results were recorded.

Analytical data of newly synthesized azo coumarine analogues:

Di] 3-(2-(pyrimidin-2-yl)diazenyl)-4-hydroxy-2H-[illegible]-2-one : Colour: Pale Brownish; Yield: 84%; m.p. 164-166^{0}C ;

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist. Parbhani

Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Dist. Parbhani Estd. 1983

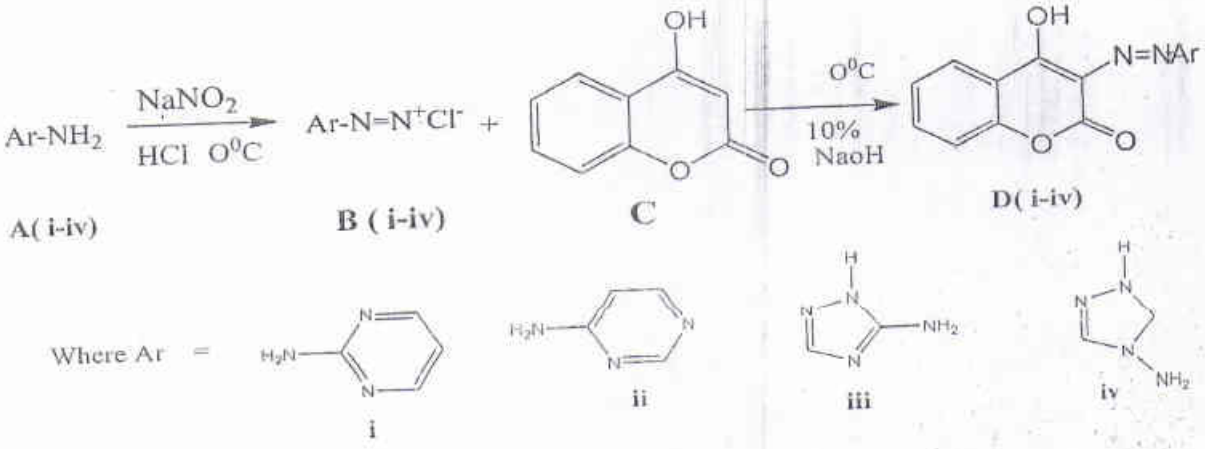
PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

The in vitro biological screenings of the investigated compounds were tested against the bacterial species by agar cup method and fungal species by the poison plate method.

Procedure:

Amino substituted hetero aryl compounds **(A i-iv)** (5mmol) were dissolved in 8ml water and 5ml conc. HCl, mixture is heated until amine hydrochloride is completely dissolved. $NaNO_2$ (5mmol) solution was prepared by dissolving it in minimum quantity of water and kept both the reaction mixture in ice bath for cooling. When these mixtures attain 0-5^{0}C temperature then $NaNO_2$ solution was added to the Amino substituted heteroaryl solution drop-wise with vigorous stirring. Near 0^{0}C temperature was maintained throughout the reaction. After the complete addition reaction mixture was kept in ice bath for 15 minutes with occasional stirring.

The diazotized reaction mixture **(B i-iv)** was then poured in ice cooled solution of 4-hydroxy coumarine **(C)** (5mmol) in 25 ml of 10% sodium hydroxide solution. This mixture was allowed to stand (0-5^{0}C) for 2 hours and then filtered. The crude product thus obtained were dried and recrystallized from acetic acid to give the corresponding compounds **(D i-iv)**.



Reaction Scheme

Antibacterial Activity

The antibacterial activity was measured by agar cup method[9]. The bacterial strains used as test organism were Escherichia coli and Staphylococcus aureus and Salmonella typhi as a gram negative

Co-ordinator IQAC Shri Guru Buddhiswami Mahavidyalaya Purna (Jn) Dist. Parbhani

PRINCIPAL Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Dist.Parbhani

IR (KBr,cm^{-1}): 3600-3200 (broad phenolic ν_{OH}), 1732 ($\nu_{C=O}$) of lactone, 1520 ($\nu_{N=N}$), 1560 and 1492 aromatic ($\nu_{C=C}$), 1334 (ν_{C-O}) phenolic-OH); 1226 (ν_{C-N}) Pyrimidine.

^{1}HNMR (δ, ppm): 7.28-8.06 m, 4H (Ar-H of coumarin moiety), 16.75 (S,1H, O–H); 7.40-8.80 m 3H (Ar-H of pyrimidine moiety);

^{13}CNMR(δ, ppm): 101.6 for C^3, 178.83 for C^4, 159.85.4 for lactone carbon, 136-115.56 for aromatic carbons of coumarin and 158 for C^2 of pyrimidine, 157.2 for C^4/ C^6, 120.3 for C^5 of pyrimidine.

Mass Spectra: $[M^+] = 267$

Dii] 3-(2-(pyrimidin-4-yl)diazenyl)-4-hydroxy-2H-chromen-2-one : Colour: pale brownish; Yield: 83%; m.p. 161-163^{0}C ;

IR (KBr,cm^{-1}): 3600-3200 (broad phenolic ν_{OH}), 1740 ($\nu_{C=O}$) of lactone, 1512 ($\nu_{N=N}$), 1555 and 1492 aromatic ($\nu_{C=C}$), 1338 (ν_{C-O}) phenolic-OH); 1230 (ν_{C-N}) pyrimidine.

^{1}HNMR (δ, ppm): 7.28-8.06 (m 4H, Ar-H of coumarin moiety), 16.75 (S,1H, O–H); 9.3 (S 1H), δ 8.8 (d 1H), 7.4 (d 1H).

^{13}CNMR(δ, ppm): 102 for C^3, 158.30. for lactone carbon, 176 for C^4, 130-117 for aromatic carbons of coumarin, and 158.6 for C^2 of pyrimidine, 121 C^3, 176 C^4, 158 C^6 of pyrimidine.

Mass Spectra: $[M^+] = 267$

Diii] 3-(2-(2H-1,2,4-triazol-3-yl)diazenyl)-4-hydroxy-2H-chromen-2-one : Colour: yellowish; Yield: 78%; m.p. 184-186^{0}C ;

IR (KBr,cm^{-1}): 3600-3300 (broad phenolic ν_{OH}), 1748 ($\nu_{C=O}$) of lactone, 1570 ($\nu_{N=N}$), 1550 and 1485 aromatic ($\nu_{C=C}$), 1340 (ν_{C-O}) phenolic-OH); 1228 (ν_{C-N}) triazole.

^{1}HNMR (δ, ppm): 7.20-8.32 (m 4H, Ar-H of coumarin moiety), 16.75 (S,1H, O–H); 13.0 (S 1H), 8.4 (S 1H)

^{13}CNMR(δ, ppm): 104 for C^3, 160.30. for lactone carbon, 174 for C^4, 130-117 for aromatic carbons of coumarin, 150.45 for C^2 and 151.6 for C^3 of triazole.

Mass Spectra: $[M^+] = 256$

Div] 3-(2-(1,5-dihydro-1,2,4-triazol-4-yl)diazenyl)-4-hydroxy-2H-chromen-2-one : Colour: yellowish; Yield: 81%; m.p. 181-183^{0}C ;

IR (KBr,cm^{-1}): 3600-3300 (broad phenolic ν_{OH}), 1742 ($\nu_{C=O}$) of lactone, 1575 ($\nu_{N=N}$), 1565 and 1488 aromatic ($\nu_{C=C}$), 1345 (ν_{C-O}) phenolic-OH); 1230 (ν_{C-N}) triozole.

^{1}HNMR (δ, ppm): 7.28-8.06 (m 4H, Ar-H of coumarin moiety), 16.75 (S,1H, O–H); 4.10 (S 2H), 7.50 (S 1H), 7.0 (S,1H).

^{13}CNMR(δ, ppm): 102 for C^3, 158.30. for lactone carbon, 176 for C^4, 130-117 for aromatic carbons of coumarin, 148.83 for C^2 and [illegible] of triazole.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Sanstha's Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Estd.1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

Mass Spectra: [M^+] = 257.

Result and Discussion :

The scheme of reaction approaching to the target heterocyclic azo compounds is outlined above. In present investigation we report newly synthesized four heterocyclic azo copounds. They were prepared by coupling 4-hydroxy-2H-chromen-2-one with diazotized heterocyclic amines. The products formed were recrystallized in ethanol and purity was tested by TLC. Different heterocyclic amines were firstly undergo diazotization by the action of sodium nitrate at 0-5 ^{0}. This diazotised mixture produces N_2^+ as strong electrophile which activates the coupling reaction with 4-hydroxy coumarine[12].

The Characterization of the synthesized compounds were done with IR, ^{1}HNMR, ^{13}CNMR techniques. The significant peaks observed in the spectra are summarized above.

The IR spectra of compound showed high intensity band observed at 1520-1575 cm^{-1} is assigned to ν(N=N) vibration suggesting the presence of N=N[13] while Broad weak band around 3600-3200 cm^{-1} is assigned to H bonded –OH in the compound. The band at 1565-1485 cm^{-1} is assigned to the combination of ν(C=C) of the aromatic ring. A high intensity band in the region 1220-1240 cm^{-1} is assigned to ν(C-N) vibration and 1748-1732 cm^{-1} for lactone carbonyl[14].

The ^{1}H NMR spectra of compound revealed singlet for H at δ15 ppm assigned to phenolic OH group[15]. Peaks between δ7.30-7.00 ppm are assigned to aromatic protons of 4-hydroxy coumarine while m (δ 9.3-7.4 ppm) indicates aromatic proton from heterocyclic amines. C^{13}NMR showed peaks between δ 110 to 175 ppm for 4 hydroxy coumarine moiety while between δ120 to 160 ppm for aromatic carbon of pyridine group. Assignment given to other peaks observed in ^{1}HNMR, ^{13}CNMR spectra and also molecular ion peaks in mass spectra justifies the structures of compounds.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Sanstha's Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Estd.1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

Table : 1 Anti-Bacterial & Anti- fungal Activity

Synthesised Azo compounds	Zone of Inhibition (diameter in mm)				Growth of Fungi				
	B. subtilis	E. coli	S. typhi	S. aureas		A. flavus	P.chrys ogenum	A. niger	F.mone liforme
Penicillin (Refernce)	14	24	18	21	**Gresiofulvin (Reference)**	-	-	-	-
D(i) 3-(2-(pyrimidin-2-yl) diazenyl)-4-hydroxy-2H-chromen-2-one	15	16	13	20	D(i)	+	++	-	++
D(ii) (3Z)-3-(2-(pyrimidin-4-yl)diazenyl)-4-hydroxy-2H-chromen-2-one	16	14	12	18	D(ii)	-	++	-	+
D(iii) 3-(2-(2H-1,2,4-triazol-3-yl) diazenyl)-4-hydroxy-2H-chromen-2-one	17	15	14	19	D(iii)	+	+	-	+
D(iv) 3-(2-(1,5-dihydro-1,2,4-triazol-4-yl)diazenyl)-4-hydroxy-2H-chromen-2-one	19	19	20	17	D(iv)	-	+	-	+

Moderate growth (++), Reduced growth (+) and No growth (-) of fungi

The heterocyclic azo compounds synthesized were evaluated for anti-bacterial and anti-fungal activity with different strains of bacteria and fungi. Results are shown in Table-1. The compounds D(i), D(ii), D(iii) and D(iv) were found to moderate to weak activities against all bacterial stains. All compounds have shown lesser activity against *E. coli, S.aureus and B. subtilis* compared with penicillin taken as standard. The activity of D (iv) compound was higher in comparison and has also shown good activity against *S. typhi* and fungi. Antifungal activity observed against *Aspergillus* species was encouraging in comparison with *Penicillium chrysogenum and Fusariummoneliforme*. However, compounds have reduced the growth of these fungi.

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Sanstha's Shri Guru Buddhiswami Mahavidyalaya Purna (Jn) Estd.1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani

References

1. R.S. Abromov, D. L. Bankoort, *J. Phys. Chem.*, 31(1), pp-23, **(1993).**
2. P.S. Jogi, S.S. Shende 7 J. S. Meshroom, *Int. Jour. Res. Bio. Agri & Tech.*, 1, pp 217-223, **(2015).**
3. A. A. H. Kadhum, A. A. Al-Amiery , A. Y. Musa and A. B. Mohamad, *Int. J. Mol. Sci.*, 12, pp 5747-5761, **(2011).**
4. M.A Bhat, Mohammed, A., Al-Omar, N. Siddiqui., *Der Pharma Chemica*, 2(2), pp 1-10, **(2010)**.
5. A. H. Shridhar1, J. Keshavayya1, H. Joy Hoskeri and R. A. Shoukat Ali, *Inter. Res. Jour. of Pu. & App. Chem.*, 1(3), pp 119-129, **(2011).**
6. G.B. Bagihalli, P. G. Avaji, S. A. Patil, P. S. Badami, *Euro. Jour. of Med. Chem.*, 43, pp 2639-2649, **(2008).**
7. L. Pochet, C. Doucet, M. Schynts, N. Thierry, N. Boggeto, B. Pirotte,K.Y. Liang, B. Masereel, P. de Tulio, J. Delarge, M. Reboud-Ravaux, *J. Med. Chem.*, 39, pp 2579, **(1996)**
8. S. Kostova, P. Raleva, Genova, R. Argirova, *Bioinorg. Chem. Appl.*, pp1-9, (2006).
9. Irena Kostova, *Curr. Med. Chem. Anti-Cancer Agents*, 5, pp 29-46, **(2005)**.
10. V. Sharma, N.Chitranshi, A. K. Agarwal, *Int. J. Med.Chem.*, 1, pp 1-31, **(2014)**.
11. Hong-Yan Guoa, Zheng-Ai Chenb, Qing-Kun Shena and Zhe-Shan Quanb, *J. Enz. Inhi. Med.Chem.*, Vol. 36(1), pp 1115–1144, **(2021).**
12. P. Sudhir Kumar, G. Ghosh, S. K. Rout and D. Paul, *Rasayan J. Chem.*, Vol. 6, No.2 pp147-152, **2013.**
13. J. O. Otutu, *IJRRAS*, 15 (2), pp 292-296, **2013.**
14. M. V. Girgaonkar and S. G. Shirodkar, *J. Chem. and Pharm. Res.*, 4(1), pp 260-264, **2012.**
15. N. A. Alrazzak, S. A. Aowda, A. J. Atiyah, J. Uni. Baby., P. App. Sci., 26 (6), pp 234-252, **(2018).**

Co-ordinator
IQAC
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn) Dist. Parbhani - 431511 (M.S.)

Shri Guru Buddhiswami Shikshan Prasarak Sanstha's Shri Guru Buddhiswami Mahavidyalaya Purna (Jn.) Estd.1983

PRINCIPAL
Shri Guru Buddhiswami Mahavidyalaya
Purna (Jn.) Dist.Parbhani