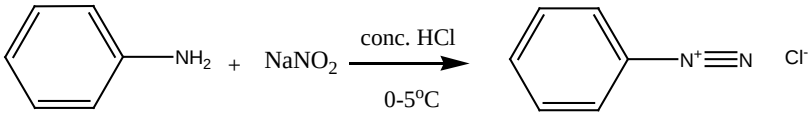
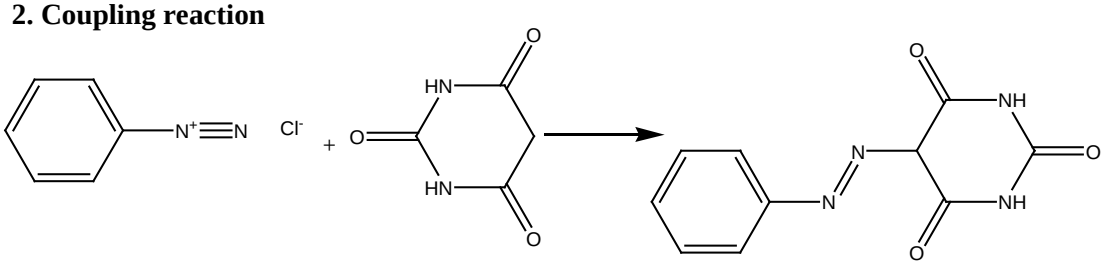
1. Diazotization reaction



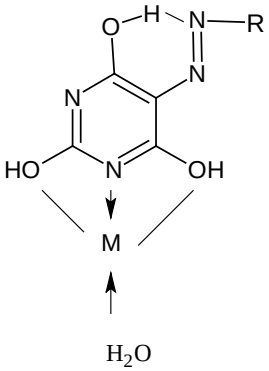
Benzene Diazonium chloride

2. Coupling reaction



Barbituric acid 5-[phenylazo]barbituric acid

3. Metal complexation



H_2O

R=Aniline, Anthranilic acid, 2-aminopyrimidine

M= Co (II), Ni (II), Cu (II)

Figure 1: Schematic diagram of synthesis of pyrimidine derivatives

4. Results& Discussion

The percentage yield of synthesized compounds and time taken by the reactions was recorded (Table 1and Table 2).

Table 1: Percentage yield of synthesized compounds

Compound	*Percentage yield (%)*	
	Conventional	Microwave
L_1 Cu	35%	47%
L_1 Ni	65%	74%
L_1 Co	57%	75%
L_2 Cu	31%	45%
L_2 Ni	26%	39%
L_2 Co	40%	55%
L_3 Cu	26%	33%
L_3 Ni	55%	69%
L_3 Co	44%	52%

Table 2: Reaction time of compounds

Compound	*Time(s)*	
	Conventional	Microwave
L_1Cu	7200	40
L_1Ni	7200	36
L_1 Co	7200	38
L_2 Cu	7200	30
L_2Ni	7200	47
L_2Co	7200	30
L_3Cu	7200	39
L_3Ni	7200	40
L_3 Co	7200	40

Melting points could not be recorded because these compounds decompose upon heating upon reaching a certain limit.

UV spectra were recorded within the range 200-600nm on Hitachi U-2800 spectrophotometer.

Table 3: UV/VIS data of synthesized compounds

Compound	*Conventional* λ_{max} *(nm)*	*Microwave* λ_{max} *(nm)*
L_1 Cu	422	421
L_1 Ni	397	396
L_1 Co	395	398
L_2 Cu	529	530
L_2 Ni	470	469
L_2 Co	470	473
L_3 Cu	583	580
L_3 Ni	569	570
L_3 Co	521	520

UV-VIS data for metal complexes synthesized by both conventional and microwave irradiation is in close approximation with each other. Low values of λ_{max} are due to $\pi - \pi^*$ and higher values are due to n-π^* transitions (table-3). The UV/Vis data of ligands showed absorption bands within the range of 290nm and 285nm respectively. On the other hand the complexes show decreased absorbance as a result of chelation.

FTIR spectra of all synthesized compounds were recordedonMidac USA M-2000 FTIR Spectrophotometer. (Table 4a, 4b). FTIR spectra for all synthesized compounds showed approximately similar values for conventional and microwave-assisted method. The IR bands at region of 3400 – 3500 cm^{-1} are due to presence of intermolecular hydrogen bond, strong bands at 16650 – 1750 cm^{-1} region are assigned to C==O, 1445–1500 cm^{-1} are due to C==N bonding and band at 400-550 show M–O bonding in metal complexes.The FTIR spectral data shows a band of metal-oxygen and metal-nitrogen bond in all the metal complexes at the range of 400-500 cm^{-1} which was not present in the ligands indicating the formation of metal complexes.

Mass spectra of all metal complexes synthesized by microwave irradiation method were taken by **GCMS Schimadzo QP-2010 Spectrometer**. Mass spectra of all metal complexes exhibited their base peaks. Peaks at 169 and 43 are evident due to fragmentations of N==N and M–O bond.

Table 4 (a): FTIR data of compounds synthesized by conventional method

Compound	*Conventional* *Wave number (cm^{-1}) Absorption Intensity*
L_1 Cu	476.04, 540.22, 601.37, 772.51, 839.15, 1137.94, 1201.22, 1268.44, 1306.46, 1383.72, 1463.73, 1516.17, 1629.45, 3219.66, 3411.82, 3471.30
L_1 Ni	490.17, 599.95, 1121.83, 1204.21, 1396.36, 14499.91, 1544.78, 1625.50, 2347.58, 2921.64, 3262.34, 3420.798
L_1 Co	470.50, 607.66, 768.65, 1116.61, 1392.84, 1549.588, 1617.85, 1715.50, 2356.0531, 3211..953, 3449.89
L_2 Cu	610.650, 679.724, 770.167, 1384.673, 1508.868, 1636.334, 2343.231, 2624.747, 3409.876, 3607.996, 3798.891, 3838.536, 3957.735
L_2 Ni	706.31, 759.48, 1396.77, 1625.65, 1710.63, 2343.23, 3304.44, 3491.946, 3589.176, 3740.94, 3782.11, 3830.70, 3876.049, 3971.572
L_2 Co	549.74, 833.77, 1200.51, 1383.68, 1516.29, 1620.43, 3404.09, 3448.85, 3737.59, 3782.74, 3822.69, 3886.95, 3950.94
L_3 Cu	426.92, 496.25, 837.45, 1376.86, 1499.97, 1641.63, 1738.48, 3850.45, 3909.42
L_3 Ni	408.02, 442.76, 515.59, 829.98, 1163.30, 1261.20, 1433.01, 1512.84, 1655.34, 1746.04, 3252.66, 3784.18
L_3 Co	745.99, 833.10, 1162.75, 1261.68, 1514.09, 1653.78, 1742.45, 3483.23, 3559.47, 3882.86

Table 4 (b): FTIR data of compounds synthesized by MW

Compound	*Microwave* *Wave number (cm^{-1}) Absorption Intensity*
L_1Cu	471.04, 516.83, 773.17, 1239.85, 1378.55, 1428.81, 1592.41, 1664.64, 1724.01, 260319, 3271.97, 3442.92
L_1Ni	510.85, 605.25, 1129.90, 1273.18, 1388.177, 1505.18, 1617.39, 2353.996, 2721.74, 3182.668, 3403.36
L_1Co	453.096, 599.455, 1122.98, 1199.51, 1389.09, 1600.94, 2359.017, 3432.98
L_2Cu	610.65, 679.72, 770.17, 1384.67, 1508.9, 1636.33, 2343.23, 2624.75, 3409.9, 3607.99, 3798.9, 3838.54, 3957.73
L_2Ni	690.41, 764.70, 1390.98, 1614.96, 1716.09,2963.29, 3554.397, 3585.07, 3675.16, 3721.788, 3775.94, 3847.18, 3901.668, 3967.639
L_2Co	545.27, 698.976, 759.18, 840.16, 906.899, 969.13, 1027.83, 1069.43, 1369.994, 1447.86, 1493.01, 1600.68, 1805.70, 1873.19, 1945.88, 2343.23, 2851.21, 2927.42, 3026.61, 3061.01, 3743.87
L_3Cu	426.92, 496.25, 837.45, 1376.86, 1499.97, 1641.63, 1738.48, 3850.45, 3909.42
L_3Ni	408.02, 442.76, 515.59, 829.98, 1163.30, 1261.20, 1433.01, 1512.84, 1655.34, 1746.04, 3252.66, 3784.18
L_3Co	745.99, 833.10, 1162.75, 1261.68, 1514.09, 1653.78, 1742.45, 3483.23, 3559.47, 3882.86

The antibacterial evaluation of compounds synthesized was studied with the stains of *Bacillus subtilis*, both the ligands and their metal complexes showed antibacterial activity.

The antifungal activity was evaluated with the culture of *Aspergillus Niger*. All the compounds showed antifungal activity at concentrations of 50µg/ml and 25µg/ml.

Inhibition zone of bacterial growth were measured in cm. All metal complexes synthesized by microwave irradiation method showed good activity against *Bacillus subtilis* bacterium. The activity results showed that activity of these compounds was due to coordination of mixed ligands to metal ions. Nickel complexes showed maximum activity with all ligands (L_1, L_2 and L_3) as compared to other metal complexes (Table-5).

All ligands and their metal complexes (Cu, Ni, Co) solutions of different concentrations were tested against fungus *Aspergillus Niger*.. All compounds gave positive results in higher concentrations (50 mg/mL, 25 mg/mL and 12.5 mg/mL).(Table 6a, 6b).

Table 5: Inhibition zone showed by bacterial species

Compound	*Inhhibition zone (cm)*
L_1Cu	2.2
L_1Ni	2.8
L_1Co	3.0
L_2Cu	2.5
L_2Ni	3.4
L_2Co	2.5
L_3Cu	2.3
L_3Ni	3.1
L_3Co	2.9

Table 6(a): Antifungal activity of compounds

Compound	*Synthesized dilutions*		
	50mg per ml	*25mg Per ml*	*12.5mg Per ml*
L_1	+	+	+
L_2	+	+	+
L_3	+	+	+
L_1 Cu	+	+	-
L_1 Ni	+	+	+
L_1 Co	+	+	-
L_2 Cu	+	+	-
L_2 Ni	+	+	+
L_2 Co	+	+	+
L_3 Cu	+	+	+
L_3 Ni	+	+	+
L_3 Co	+	+	+

Table 6(b): Antifungal activity of compounds

Compound	*Synthesized dilutions*		
	6.25mg Per ml	*3.125mg Per ml*	*1.575mg Per ml*
L_1	-	-	-
L_2	-	-	-
L_3	-	-	-
L_1Cu	-	-	-
L_1Ni	-	-	-
L_1Co	-	-	-
L_2Cu	-	-	-
L_2Ni	-	-	-
L_2Co	-	-	-
L_3Cu	-	-	-
L_3Ni	-	-	-
L_3Co	_	_	-

5. List of abbreviations

Sr. No.	*Compound Name*	*Abbreviations*
1.	5-[phenylazo] pyrimidine 2, 4, 6-trione	L_1
2.	5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione	L_2
3.	5-(pyrimidinil-2-azo) barbituric acid	L_3
4.	Copper complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione	CuL_1
5.	Nickel complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione	Ni L_1
6.	Cobalt complex of 5-[phenyl azo] pyrimidine 2, 4, 6-trione	Co L_1
7.	Copper complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione	Cu L_2
8.	Nickel complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione	Ni L_2
9.	Cobalt complex of 5-[o-carboxy phenyl azo] pyrimidine 2, 4, 6 trione	Co L_2
10.	Cu-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione	Cu L_3
11.	Ni-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione	Ni L_3
12	Co-complex of 5-[2-pyrimidinylazo) pyrimidine 2, 4, 6 trione	Co L_3

6. Conclusion

This reported research work was designed to synthesize bioactive pyrimidine azo compound and their metal complexes by using simple and eco-friendly synthetic methods and their comparison with that of conventional ones. Reduced reaction time, increased reaction rate and improved yield with high purity makes this protocol as competent and trouble-free synthetic strategy.

The structural elucidation of synthesized compounds were carried out via FTIR, UV/Vis, GC-MS and the antimicrobial (antibacterial and antifungal) activities of compounds were also studied. Although, compounds synthesized by both methods were in close agreement in the terms of their outputs. But microwave-assisted technique has demonstrated enormous advantages over the conventional ones and can be opted as first choice by synthetic chemists.

7. Future Scope

Microwave-assisted synthesis has been proved an efficient synthetic route to synthesize a large number of organic compounds. Its advantages over conventional ones are a breakthrough in organic synthesis. By using this eco-friendly methodology, better results can be produced in reduced time and cost. It can be used as key source to synthesize a large number of bioactive compounds on large scale.

8. Acknowledgement

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References

[1] J. A. Joule, K. Mills, Oxford Wiley, pp. 250, 2010.
[2] W.C.Cutling, "Handbook of Pharmacology", 3rdEdition, Meredith Company, 1967.
[3] S. Wang, S. Shen, H. Xu, "Synthesis, spectroscopic and thermal properties of a series of azo metal chelate dyes", Dyes and Pigments, 44, pp. 195-198, 2000.
[4] Y. Jinghua,Z. Guanxing, F. Huaxue, 26(12),15-19, 1998.
[5] E. D. Neas, M. J. Collins, "Introduction to microwave sample preparation theory and practice", American Chemical Society, 7-32, 1988.
[6] M. S. Masoud,A. E. Ali, A. M. Shaker, G. S. Elasala, "Synthesis, computational, spectroscopic, thermal and antimicrobial activity studies on some metal–urate complexes" SpectrochimicaActa,90,93-108, 2012.
[7] F. Karci,, N. Sener, M. Yamak, I. Sener, A. Demircali, "The synthesis, antimicrobial activity and absorption characteristics of some novel heterocyclic disazo dyes", dyes and pigments, 80(1) pp. 47-52, 2009.
[8] R. Gup, E. Giziroglu, B. Kirkan, "Synthesis and spectroscopic properties of new azodyes azo-metal complexes derived from barbituric acid and aminoquinoline", dyes and pigments, 73(1),pp. 40-46, 2007.
[9] F. Karci, "The synthesis and solvatochromic properties of some novel heterocyclic disazo dyes derived from barbituric acid." dyes and pigments, 77(2), pp. 451-456, 2008.
[10] M. Tyagi, S. Chandra, "Synthesis and spectroscopic studies of biologically active tetraazamacrocyclic complexes of Mn(II), Co(II), Ni(II), Pd(II) and Pt(II)",Journal of Saudi Chemical Society, 2011.

Author Profile

Dr. Bushra Khan received the M. Sc and Ph. D degree in Analytical chemistry from Punjab University, Lahore Pakistan. Now she is a professor, Dean of natural sciences and head of Chemistry department of Lahore College for women university Lahore, Pakistan. She is teaching and conducting research of graduate and post graduate students. Her research papers have been published in various journals.

Dr. Shahana Ehsan received M. Sc and Ph. D degree from Punjab University and Lahore College for Women University Lahore, Pakistan respectively. Now she is Assistant Professor and teaches graduate and post graduate students and conducting their research.

Aneeqa Ishfaq received her BS and MS degree from Lahore College for Women University Lahore, Pakistan. Now she is PhD scholar.

Tahira Saghir received her MS degree from Lahore College for Women University Lahore, Pakistan. Now she is PhD scholar.

Sehrish Ghafoor received her BS (Hons) and MS degree from Lahore College for Women University Lahore, Pakistan. She is an alumni of Lahore College for Women University.