SYNTHESIS CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF 1, 2, 3- TRIAZINE DERIVATIVES

Supriya Patel*, Deepak Kumar Basedia, Dr. B K Dubey, Bhushan Korde, Vivek Singh Thakur, Sandra Gotam, Sakshi Patel

Technocrats Institute of Technology Pharmacy, Bhopal M P

***Corresponding author**

Supriya Patel

E-mail: bpart0001@gmail.com

Technocrats Institute of Technology Pharmacy, Bhopal M P

ABSTRACT

Triazines have a high significance in the field of medicinal chemistry with broad-spectrum of pharmacological activities. Thus triazine can be useful for design and formation of novel drugs. Some triazine derivatives are currently being evaluated in clinical trials. Triazine may lead to potent type drugs with no or fewer side effects as compared to presently available pharmacological agents. In this research some new type of antimicrobial agent were synthesized 1,2,3 Triazine derivatives. The 1,2,3 Triazine derivatives linked with Schiff bases as antimicrobial agent. 1,2,3-Triazine derivatives were synthesized by cyclization of (E)-N-(2-(3,3-diisopropyltriaz-1-en-1-yl)benzyl)acetamide in presence of TFA then formed Schiff base by reacting 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone with different substituted amines. Characterization of newly synthesized triazine compounds were formed as Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f - value. Structure of synthesized Triazine derivatives were elucidated using spectrogram data as well as physical method e.g Elemental analysis, FT-IR, ^{1}H NMR. Synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds were also characterized. Biological evaluation of synthesized trazine derivatives was performed against Gram positive (*B.*

subtilis) and Gram negative bacteria (*E. coli*). Antimicrobial activities were estimated by disk diffusion method. Firstly, minimum inhibitory concentration (MIC) values obtained. All five synthesized compounds were less effective against both bacterial strains except **Triazine-2** and **Triazine-5** were more effective against *E. Coli.* and concluded that further design may prove an alternative and very useful and fruitful in the discovery of new antimicrobial activity in comparison to standard drugs.

KEYWORDS: Triazines, Synthesis, Antimicrobial, Multidrug Resistant, Schiff Bases

INTRODUCTION

Triazines have a high significance in the field of medicinal chemistry with broad-spectrum of pharmacological activities[1]. Thus triazine can be useful for design and formation of novel drugs. Some triazine derivatives are currently being evaluated in clinical trials[2]. Triazine may lead to potent type drugs with no or fewer side effects as compared to presently available pharmacological agents[3].

Fusion of 1,2,3-triazine with heterocyclic moiety like indolo-benzo, pyridine, indole, thieno, pyrazolone, triazole and 1,2,4-triazine moieties have been reported to show improved chemical and pharmacological activities[4]. The heterocyclic system containing indole and triazine rings alone and in fused form together exhibit significant antitumor activity against leukaemia and broad spectrum antimicrobial activity, while presence of benzene in the heterocyclic system with 1,2,3-triazine shows significant antitumor activity in the various cancer cell lines[5,6]. Since the 1980s, a re-emergence of tuberculosis has occurred that is often multidrug resistant (MDR) and enhanced by human immunodeficiency virus infection[7]. The severity and difficulty in treating MDR strains necessitates the use of several, sometimes six to seven different drug[8].

In this research we want to synthesize some new type of antimicrobial agent so we choose 1,2,3 Triazine derivatives. The selection 1,2,3 Triazine derivatives as antimicrobial agent because 1,2,3 Triazine derivatives already reported for antibacterial antifungal activity and Schiff bases are well known for antimicrobial activity. These are basic points which provide us a strong rational for the research i.e. synthesis and antimicrobial screening of 1,2,3 Triazine and their derivatives.

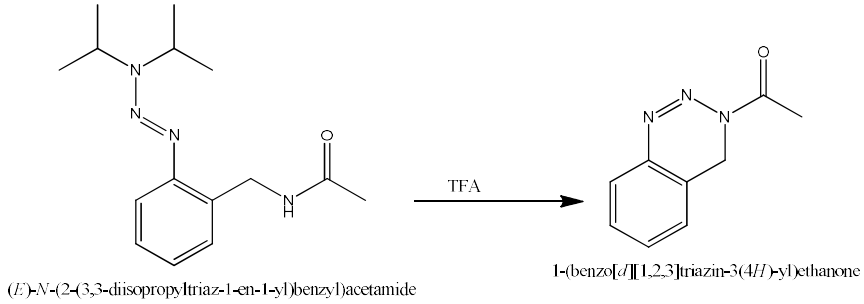
MATERIALS AND METHOD

(E)-N-(2-(3,3-diisopropyltriaz-1-en-1-yl)benzyl)acetamide, Methylamine, Ethylamine, Benzylamine, and 1,4 benzene diamine were purchased from Sigma Aldrich Co. and other reagents used belongs to lab reagent great.

Methods of synthesis of 1, 2, 3 Triazine

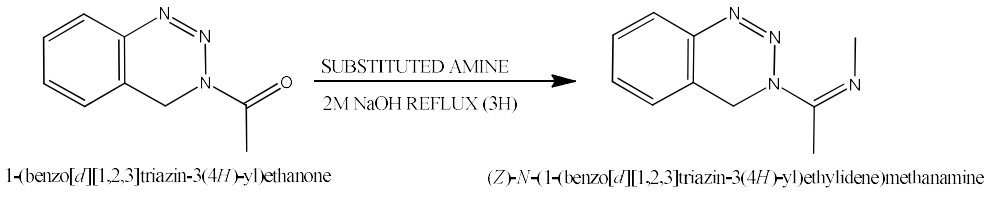
Synthesis of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone

(E)-N-(2-(3,3-diisopropyltriaz-1-en-1-yl)benzyl)acetamide (0.001 mol) was taken in a flask and added Trifluoroacetic acid (15ml) stirred on a magnetic stirrer at 50-60°C for 50-60 minutes. It was then poured into crushed ice. The solid separate out was filtered and washed. It was recrystalized with suitable solvent.



Synthesis of Schiff Bases from 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone using different substituted amines

(a) Synthesis of (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)methanamine (TRIAZINE-1): methylamine (15ml, 10mmol) was taken in a beaker with 2M NaOH (10mL) and to it was added a solution of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone (1.75g, 10mmol) in



(b) Synthesis of (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene) ethanamine (TRIAZINE-2):ethylamine (15ml, 10mmol) was taken in a beaker with 2M NaOH (10mL) and to it was added a solution of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone (1.75g, 10mmol) in

methanol (50mL) drop wise. The mixture was heated under reflux for three hours. After cooling, the mixture was filtered and evaporated under reduced pressure. The product obtained, washed with acetone and dried.

(c) Synthesis of (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)-1-phenylmethanamine (TRIAZINE-3): benzylamine (15ml, 10mmol) was taken in a beaker with 2M NaOH (10mL) and to it was added a solution of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone (1.75g, 10mmol) in methanol (50mL) drop wise. The mixture was heated under reflux for three hours. After cooling, the mixture was filtered and evaporated under reduced pressure. The product obtained, washed with acetone and dried.

(d) Synthesis of (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)aniline(TRIAZINE-4): phenylamine (15ml, 10mmol) was taken in a beaker with 2M NaOH (10mL) and to it was added a solution of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone (1.75g, 10mmol) in methanol (50mL) drop wise. Mixture was heated under reflux for three hours. After cooling, mixture was filtered and evaporated under reduced pressure. The product obtained, washed with acetone and dried.

(e) Synthesis of (Z)-N1-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)benzene-1,4-diamine (TRIAZINE-5): 1,4 benzene diamine (15ml, 10mmol) was taken in a beaker with 2M NaOH (10mL) and to it was added a solution of 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone (1.75g, 10mmol) in methanol (50mL) drop wise. The mixture wa reflux for three hours. After cooling, the mixture was filtered and evaporated under reduced pressure. The product obtained, washed with acetone and dried.

Methods of Identification and Characterization

Physical Evaluation of Newly Synthesized Compound: Physical evaluation included Color, consistency; physical stat and smell were sensed by visual and other sensory organs and recorded.

Calculation of % yield: Percentage of Yield was calculated by following formula:

$$\% \textbf{ Yield } = \frac{\textbf{Practical yield}}{\textbf{Theoretical yield}} \textbf{ X 100}$$

Melting Point Determination: The melting points of the organic compounds were determined by open capillary tube method. Melting point is a valuable criteria of purity for an organic compound as a pure crystal is having definite and sharp melting point. The purity should not be assumed but must be established by observation of any changes in the melting point when the compound is subjected to purification by recrystalisation. The synthesized compounds showed a minute change in melting point after recrystallisation.

Solubility: The solubility of synthesized compounds was tested in various solvents such as water, ethanol, chloroform, ether, benzene, methanol, dimethyl formamide (DMF) and dimethyl sulphoxide (DMSO) were taken for dissolving intermediates and final products. 10 mg of each compound was weighed and added to 10 ml of each solvent individually taken in 50 ml beaker. The observation was recorded observed for different compounds.

Thin layer chromatography: T.L.C is widely used that it has become an essential technique for analyst and research workers. In thin layer chromatography the separation is carried out on a glass or plastic which is coated with a thin uniform layer of finely divided inert absorbent such as silica gel or alumina. For the preparation of the plate, at first dry glass plates were taken. After this, silica–G was prepared in the ratio 1:2 in water. After the preparation of slurry, pouring method was used for the preparation of T.L.C plate. These plates were dried first room temp, after this are dried 110°C for one hour.

After this preparation the standard and test sample were spotted on the prepared plate with the help of small bored capillary tube. After this spotting plate were allowed to dry at room temperature, and this are transferred in to the development chamber for development of the sample. When plates were kept in to the development chamber, the solvent were development. Solvent chloroform: methanol (6:4) was used then kept out and dried. After the drying the spot were detected with the help of iodine chamber and U.V chamber. After all this Rf value were calculated. With the help of Rf value we can easily differentiate between parent compound and test compound:

$$\boldsymbol{Rf\ value = \frac{Distance\ traveled\ by\ Solute}{Distance\ traveled\ by\ solvent}}$$

In all this case the distance travelled by the sample was different from the parent compound. From all this it was easily understood that test sample were different from the parent compound.

Elemental analysis: Elemental analysis of compounds was done on Vario micro elemental analyser (Elementar Germany).

FTIR spectral analysis: IR spectra of the compound were obtained from FT-IR spectrophotometer using KBr pellets, recorded on Bruker FT-IR.

^{1}H-NMR spectral analysis: ^{1}H NMR spectra of compounds was recorded on Bruker NMR spectrophotometer in deuterium- substituted chloroform as solvent of compounds and TMS (tetra methyl silane) was used as internal standard. Chemical shift was measured as delta-value (δ-value) in ppm (parts per million).

Method of *in-vitro* Antimicrobial Screening of 1,2,3 triazine derivatives

Test organisms: Standardized cultures of bacteria *Escherichia coli* (ATCC 9637) and *Bacillus subtilis* (ATCC 9372) and fungi *Candida albicans* (ATCC 10231) and *A. Niger* were obtained from the Microbiology Laboratory, NIPRD.

Antibiotic susceptibility testing on bacteria: Antibacterial activity of synthesized compounds was evaluated by the paper disk diffusion method on Mueller-Hinton agar (MHA) plates. Bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated onto MHA plates (15cm diameter). Sterile filter paper disks (diameter 6 mm) soaked in a known concentration of extracts (5000 µg/ml per disk) in DMSO were applied over each of the culture plates previously seeded with the 0.5 McFarland and 106 CFU/ml cultures of bacteria. The cultured plates were then incubated at 37°C for 18h. Paper disks soaked in a known concentration (50µl) of ciprofloxacin in distilled water as standard antimicrobials were used as positive control. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disk. For each extract, three replicate trials were conducted against each organism.

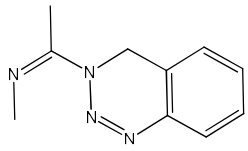
Statistical Analysis

All the values are expressed as mean standard error of mean (S.E.M.) and analyzed by one way ANOVA and posthoc Tukey multiple comparison test by employing statistical software, Graph Pad In Stat 3. Differences between groups were considered significant at $P < 0.05$ levels.

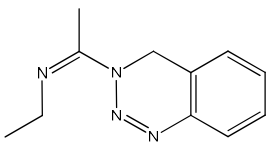
RESULTS AND DISCUSSION

Characterization and Structural Elucidation of Novel Synthesized Triazine Derivatives

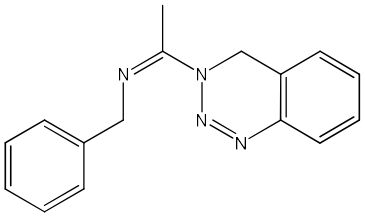
TRIAZINE-1: IUPAC Name: (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl) ethylidene) methanamine, **Molecular weight:** 188.23, Yellow, Crystalline solid, **Melting point**: 136 ^{O}C , **Yield:** 70.07%, **Solubility**: Chloroform, DMF and Methanol, **R_f:** 0.56; **Elemental analysis calculated (Found) % for $C_{10}H_{12}N_4$:** C, 63.81(63.62); H, 6.43(6.14); N, 29.77(29.51). **FT-IR (KBr): cm^{-1}** 3345 **N-H** str.; 2927 **C-H** str. (alkane); 1655 **C=N** str.(Ar), 1540 **C=C** str. (Ar); 1445 **C-H** str.; 1325 **C-N** str. **^{1}H NMR (MeOD, 500 MHz):** δ 7.35-7.20(m, 4H, CH-Ar), 3.91(s, 2H, CH-Ar), 2.18(s, 3H, CH_3), 0.73 (s, 3H, N-CH_3), ppm.



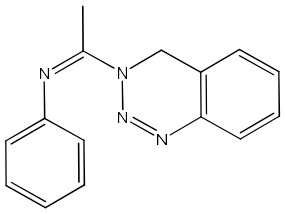
TRIAZINE-2: IUPAC Name: (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene) ethanamine; **Molecular weight**: 202.26; Yellow Crystalline solid; **Melting point**: 136-138 ^{O}C ; **Yield**: 76.27 %, **Solubility:** Chloroform, DMF and Methanol; **R_f** : 0.56; **Elemental analysis calculated (Found) % for $C_{11}H_{14}N_4$:** C, 68.75(68.32); H, 6.11(6.01); N, 25.13(25.06). **FT-IR (KBr): cm^{-1}** 3325 **N-H** str.; 2897 **C-H** str. (alkane); 1648 **C=N** str.(Ar), 1549 **C=C** str. (Ar); 1487 **C-H** str.; 1321 **C-N** str. **^{1}H NMR (MeOD, 500 MHz):** δ 7.35-7.20(m, 4H, CH-Ar), 3.91(s, 2H, CH-Ar), 2.17(s, 3H, CH_3), 1.54(q, 2H, N-CH_2), 0.92(t, 3H, J = 7.55, 5.7 N-CH_2), ppm.



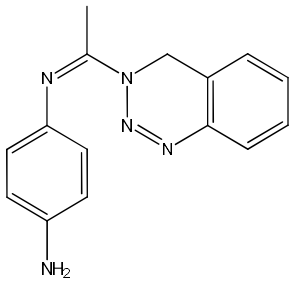
TRIAZINE-3: IUPAC Name: (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)-1-phenylmethanamine **Molecular weight**: 264.33; **Physical state**: Pale Yellow Crystalline solid; **Melting point** : 142-144^{O}C ; **Yield**: 75.17%; **Solubility:** Chloroform, DMF and Methanol; **R_f** : 0.53**; Elemental analysis calculated (Found) % for $C_{16}H_{16}N_4$:** C, 68.75(68.32); H, 6.11(6.01); N, 25.13(25.06). **FT-IR (KBr): cm^{-1}** 3312 **N-H** str.; 2811 **C-H** str. (alkane); 1648 **C=N** str.(Ar), 1548 **C=C** str. (Ar); 1498 **C-H** str.; 1313 **C-N** str. **^{1}H NMR (MeOD, 500 MHz):** δ 7.44-7.20(m, 9H, CH-Ar), 3.92(s, 2H, CH-Ar), 2.65(s, 2H, N-CH_2)2.17(s, 3H, CH_3),ppm. **MS:** 264.33



TRIAZINE-4: IUPAC Name: (Z)-N-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene) aniline; **Molecular weight**: 250.30; Yellowish Red Crystalline solid; **Melting point**: 139-141^{O}C; **Yield**: 59.33%, ; **Solubility**: Chloroform, DMF and Methanol; **R_f**: 0.51; **Elemental analysis calculated (Found) % for $C_{15}H_{14}N_4$:** C, 71.98(71.25); H, 5.64(5.28); N, 22.38(22.73).; **FT-IR (KBr): cm^{-1}** 3387 **N-H** str.; 2925, 2855 **C-H** str. (alkane); 1655 **C=N** str.(Ar), 1550 **C=C** str. (Ar); 1441 **C-H** str.; 1351 **C-N** str. ; **^{1}H NMR (MeOD, 500 MHz):** δ 7.49(t, 2H, *J*= 9.55, 10.55, CH-Ar), 7.35-7.20(m, 4H, CH-Ar), 7.08(t, 1H, *J*= 10.55, 10.8, CH-Ar), 6.99(d, 2H, *J*= 10.15, CH-Ar), 3.92(s, 2H, CH-Ar), 2.16(s, 3H, CH_3),ppm. **MS:** 250.30



TRIAZINE-5: IUPAC Name: (Z)-N1-(1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethylidene)benzene-1,4-diamine; **Molecular weight**: 265.31; Dark Yellow, Crystalline solid; **Melting point**: 142-145^{O}C; **Yield**: 73.81%; **Solubility**: Chloroform, DMF and Methanol; **R_f**: 0.61. Elemental **analysis calculated (Found) % for $C_{15}H_{15}N_5$:** C, 67.90(67.62); H, 5.70(5.11); N, 26.40(25.96). **FT-IR (KBr): cm^{-1}** 3479 **N-H** str.; 2813 **C-H** str. (alkane); 1634 **C=N** str.(Ar), 1597 **C=C** str. (Ar); 1434 **C-H** str. **^{1}H NMR (MeOD, 500 MHz):** δ 7.38(d, 2H, *J*= 6.55, CH-Ar), 7.35-7.20(m, 4H, CH-Ar), 6.67(d, 2H, *J*= 12.3, CH-Ar), 6.30(s, 2H, N-H_2), 3.91(s, 2H, CH-Ar), 2.17(s, 3H, CH_3),ppm. **MS:** 250.30



Evaluation of Antimicrobial Activity of Newly Synthesized Triazine Derivatives

Table No.1: Antimicrobial activity of Triazine Derivatives

Sample Code	Diameter of zone of inhibition (mm)			
	B. subtilis	***E. coli***	***C. Albicans***	***A. Niger***
TRIAZINE-1	16(25)	11(6.25)	20(6.25)	20(12.5)
TRIAZINE-2	12(25)	28(6.25)	18(6.25)	20(12.5)
TRIAZINE-3	9(6.25)	10(6.25)	19(6.25)	19(12.5)
TRIAZINE-4	11(6.25)	13(6.25)	20(6.25)	13(12.5)
TRIAZINE-5	13(12.5)	24(12.5)	22(6.25)	21(12.5)
Control **(C)**	-	-	-	-
Ciproflaxacin **(S)**	20(6.25)	25(6.25)	20(6.25)	25(12.5)

[a] Values in brackets are MIC values ($\mu g\ ml^{-1}$)

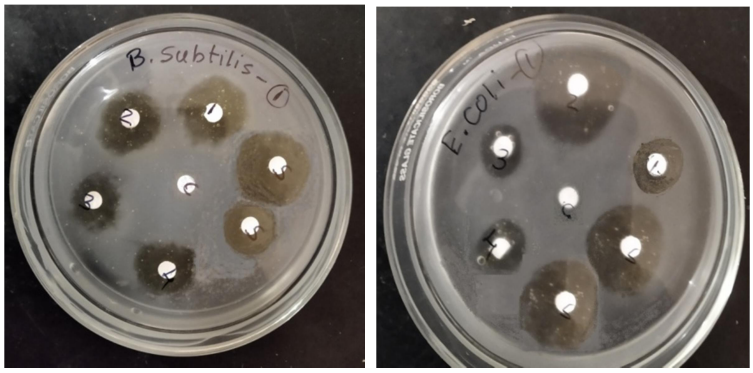


Figure 1: Antibacterial activity of Triazine Derivatives

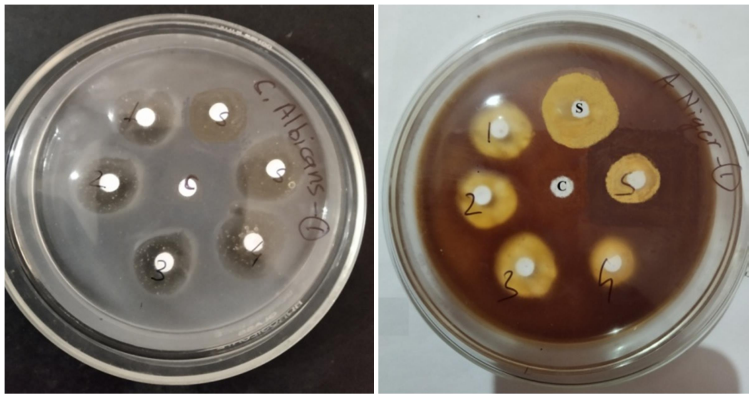


Figure 2: Antifungal activity of Triazine Derivatives

Statistical representation of pharmacological evaluation data

Antibacterial Activity

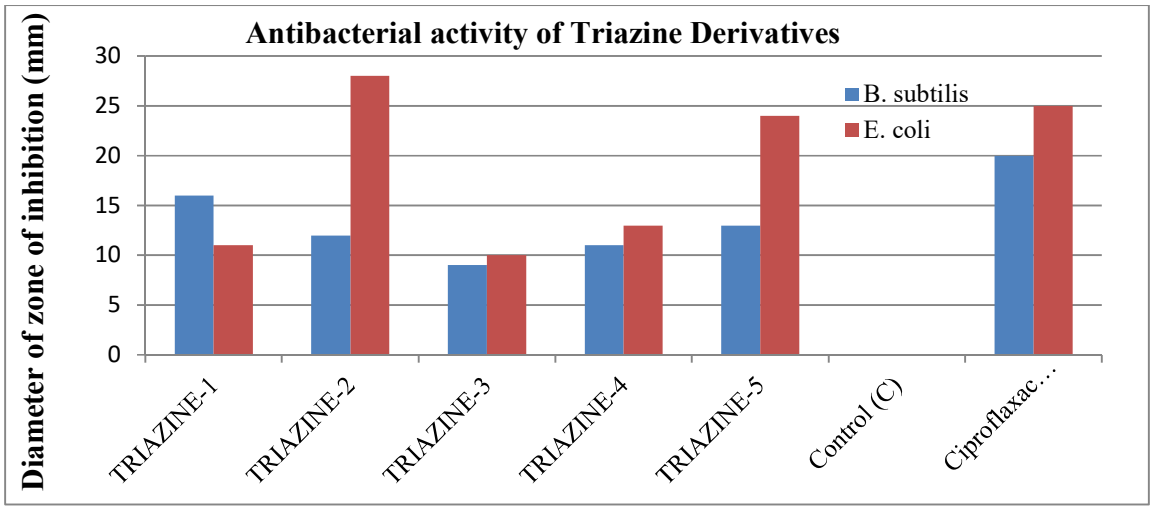


Figure 3: Antibacterial activity of extracts of Triazine Derivatives

7.3.2 Antifungal Activity

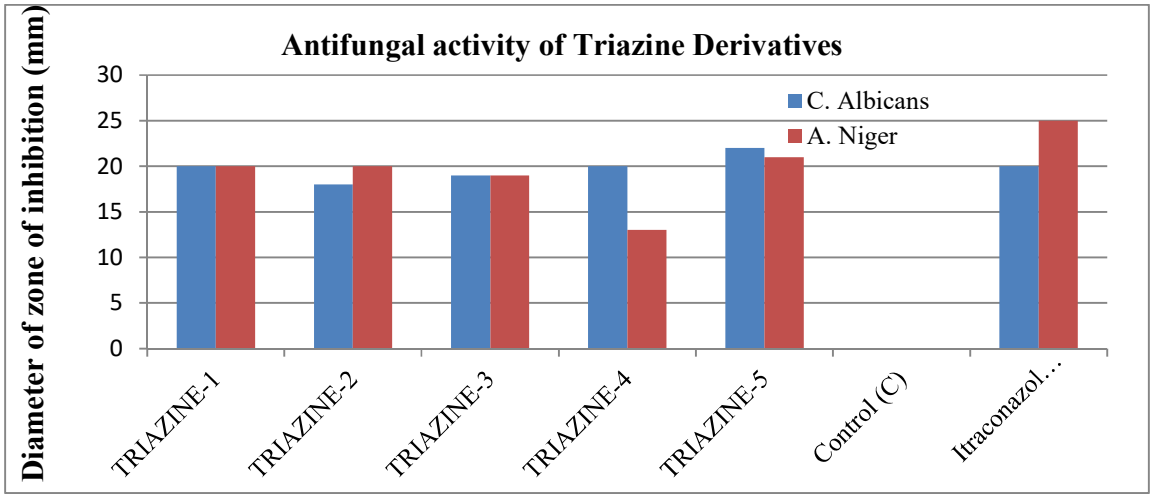


Figure 4: Antifungal activity of extracts of Triazine Derivatives

DISCUSSION

1,2,3-Triazine derivatives were synthesized by cyclization of (E)-N-(2-(3,3-diisopropyltriaz-1-en-1-yl)benzyl)acetamide in presence of TFA then formed Schiff base by reacting 1-(benzo[d][1,2,3]triazin-3(4H)-yl)ethanone with different substituted amines. Characterization of

newly synthesized triazine compounds were formed as Molecular formula, Physical state, Color, Melting point, Yield, Solubility and R_f- value. Structure of synthesized Triazine derivatives were elucidated using spectrogram data as well as physical method e.g Elemental analysis, FT-IR, ^{1}H NMR and mass. Appearance of absorption bands in compounds at 3345(-NH- N-H str.) and Schiff base at 1655 (**C=N** str.) which is present in all triazine derivative's in FT-IR spectrum clearly indicated. This fact was further supported by ^{1}H NMR spectrum, presence of peak 2.18 & 0.73 ppm of different derivative, corresponding to their structure. Moreover, synthesized compounds characterized by the TLC and melting point. In addition, by visual inspection synthesized compounds were also characterized.

Biological evaluation of synthesized trazine derivatives was performed against Gram positive (*B. subtilis*) and Gram negative bacteria (*E. coli*). Antimicrobial activities were estimated by disk diffusion method. Firstly, minimum inhibitory concentration (MIC) values obtained. Antimicrobial activities of synthesized trazine derivatives were evaluated in the term of zone of inhibition as described in tables. Triazine-1 was equally effective as itraconazole on *A. Niger,* moderately effective on *B. Subtilis* and *C. Albicans* and less effective against *E. Coli.* Triazine-2 was effective on *E. Coli* moderately effective against both fungal strains and less effective against *B. Subtilis.*Triazine-3 was moderately effective against both fungal strains and less effective against both bacterial strains. Triazine-4 was moderately effective against *C. Albicans* and less effective against both bacterial strains and *A. Niger.* Triazine-5 was effective against on *E. Coli* and *C. Albicans,* moderately effective against *A. Niger* and less effective against *B. Subtilis.* Excellent effect in comparison to standard drug possessed Triazine- 2 against *E. Coli.* All five synthesized compounds were effective against both fungal strains except Triazine-4 is less effective on *A. Niger*. All five synthesized compounds were less effective against both bacterial strains except Triazine-2 and Triazine-5 were more effective against *E. Coli.*

CONCLUSION

123-triazines are interesting groups of heterocyclic compounds exhibiting diverse pharmacological activities. They have wide range of applications starting from antimicrobial, anti-inflammatory, analgesic, antitubercular, anticancer, anti-HIV, antimicrobial to antidepressant and antihypertensive activities. Reported structure based drug design too gives an emphasis on 123-triazines moiety. These models as such for synthesis give good opportunities to

look for discovering ideal lead for antimicrobial activity. On the basis of these synthesized some new triazine derivatives then carried out test for their antimicrobial action. Further design may prove an alternative and very useful and fruitful in the discovery of new antimicrobial activity in comparison to standard drugs.

CONFLICTS OF INTERESTS

There are no conflicts of interests

REFERENCES

1. Eicher T., Hauptmann S.: The Chemistry of Heterocycles. Structure, Reactions, Syntheses and Applications. pp. 354ñ358. George Thieme Verlag, Stuttgart, New York 1995.
2. Morrison RT, Boyd RN (2009). Organic Chemistry. Chapter 27, third edition, Paula Yurkanis Bruice, University of California, Santa Barbara, Fifth impression, p. 1133.
3. Khalil AEGM, Berghot MA, Gouda MA, (2011). Design, synthesis and antibacterial activity of new phthalazinedione derivatives. Journal of the Serbian Chemical Society, 76, 329-39.
4. Patel RB, Chikhalia KH (2007). Synthesis of novel PETT analogues, 3,4-dimethoxy phenyl ethyl 1,3,5-triazinyl thiourea derivatives and their antibacterial and antiHIV studies. Journal of the Brazilian Chemical Society, 18, 312-21.
5. R. sahu, D.S. thakur and P. Kashyap “Schiff base: an overview of its medicinal chemistry potential for new drug molecules” international journal of pharmaceutical sciences and nanotechnology, vol-5, issue 3, 2012.
6. A. M. Isloor, B. Kalluraya and P. Shetty, “Regioselective Reaction: Synthesis, Characterization and Pharmacological Studies of Some New Mannich Bases Derived from 1,2,4-Triazoles,” European Journal of Medicinal Chemistry, Vol. 44, No. 9, 2009,3784-3787
7. S. Eswaran, A. V. Adhikari and N. S. Shetty, “Synthesis and Antimicrobial Activities of Novel Quinoline Derivatives Carrying 1,2,4-Triazole Moiety,” European Journal of Medicinal Chemistry, Vol. 44, No. 11, 2009, pp. 4637- 4647.
8. A. O. deSouza, F. C. S. Galetti, C. L. Silva, B. Bicalho, M. M. Parma, “Antimycobacterial and Cytotoxicity Activity of Synthetic and Natural Compounds,” Quimica Nova, Vol. 30, No. 7, 2007, pp. 1563-1566.