Vijayakumar B. *et al.* / *International Journal of Pharmacy & Therapeutics*, 3(2), 2012, 167-173.

e- ISSN 0976-0342
Print ISSN 2229-7456

International Journal of Pharmacy & Therapeutics

Journal homepage: www.ijptjournal.com

IJPT

MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF PIPERAZINE DERIVATIVES

[1]B. Vijayakumar, [2]P. Dheen Kumar, [2]D. Babuananth, [2]R. Murugan, [3]P. Gobalakrishnan, [4]M. Nishanthi

[1]Department of Pharmaceutical Chemistry, Sri Venkateswara College of Pharmacy, R.V.S Nagar, Chittoor-517127, Andhra Pradesh, India.
[2]Department of Pharmaceutical Chemistry, EJS Pillai College of Pharmacy, Nagappatinam, Tamilnadu, India.
[3]Department of Pharmaceutical Chemistry, Vagdevi College of pharmacy and research centre, Brahmadevam, Nellore-524346, Andhra Pradesh, India.
[4]Department of Pharmacognosy, Sri Venkateswara College of Pharmacy, R.V.S Nagar, Chittoor-517127, Andhra Pradesh, India.

ABSTRACT

Microwave assisted organic synthesis is an enabling technology for accelerating drug discovery and development processes. A series of novel 4-(substituted amino) phenyl)-N-phenylpiperazine-1-amine as potential biological active compounds were derived from microwave irradiation with bromoaniline and different aromatic aldehydes. All the compounds were characterized by melting point, TLC and their chemical structures were confirmed by elemental analysis, spectrometry (IR, ^{1}H NMR). The synthesized compounds were subjected for antibacterial and antioxidant activity. The synthesized compound was shown to exhibit good antibacterial activity comparable to that of standard. The antioxidant properties were evaluated by following methods: Phosphomolybdate method and reducing power assay. The compound $3a_1$ showed good antioxidant activity compared to that of standard.

Key Words:- Microwave, Piperazine, Antioxidant, Antibacterial.

INTRODUCTION

Antimicrobial agents (Tripathi KD, 2003) are the substance produced wholly or partially by micro-organism or similar substances, by chemical synthesis, which in low concentrations inhibit the growth of micro-organisms. Best antimicrobial prescribing requires an understanding of the pharmacokinetic property of the drugs, their mode of action and their spectrum of activity against infecting organisms, coupled with the clinical skills to diagnose the body system affected and most likely infecting organism. Although effective antimicrobial therapy is potentially available for all infections, the ongoing use of antimicrobial has potentiated high levels of resistance the most disturbing development of resistance has been in Staphylococci. Methicillin-resistant *Staphylococcus aureus* (MRSA) and now glycopeptides-resistant *Staph. Aureus* and *Enterococci* (GRSA, GRSE) are also there. Multidrug-resistant M.tuberculosis (MDRTB) is a similar serious threat (Roger Walker, 2003). The synthetic or naturally occurring agents, which can kill or inhibit the growth of bacterial cells, are called antibacterial agents. The goal is to limit toxicity to the host and maximize

Corresponding Author

B. Vijayakumar
Email:- vijaykumarbvk86@gmail.com

chemotherapeutic activity affecting invading microbes only. Antioxidant is a substance capable of inhibiting oxidation and this may be added for to pharmaceutical products subject to deterioration by oxidative processes. Antioxidants are among the most important candidates in controlling or preventing the free-radical reaction. An antioxidant if present in low concentration can prevent oxidation of substances like proteins, lipids and DNA. The major biological antioxidants are ascorbyl palmitate, tocopherol (vitamin E), Beta carotene, plant phenolics and thiol containing compounds (Surendra NP *et al.*, 2003).

They have been used to treat neurodegenerative disorders such as Alzeimer's and Parkisonian's disease and also cognitive dysfunction. Selegine although MAO inhibitor, may have neuroprotective effects through its antioxidants activity. Vitamin E is free radical scavenger and immune enhancer and can be combined with existing drugs for treatment of patients with HIV or AIDS. Heterocycles (Raj.K.Bansal *et al.*, 2005) containing piperazine rings are associated with a wide range of biological properties such as antimicrobial, anticancer, antihypertensive, analgesic, antidepressant, anti-inflammatory and antiheliminthic activity. Piperazine derivatives containing aryl –SO3 group has received no attention in spite of welldefined biological activities of piperazine containing compounds. Hence it was thought interesting to synthesize novel piperazine derivatives containing aryl –SO3 group. The present paper comprises the synthesis, characterization and biological evaluation of piperazine derivatives as shown in Scheme -1.

EXPERIMENTAL

MATERIALS AND METHODS

The melting points were taken by using a Thomas Hoover capillary melting point apparatus and were uncorrected. The purity of the synthesized piperazine derivatives was checked by TLC using silica gel-G 254 aluminium sheets using chloroform: Methanol (8:2) as eluent and visualized in a ultra violet chamber (Table-2). IR spectra were recorded (in KBr) on FTIR 8300 Shimadzu spectrophotometer. The ^{1}HNMR spectra were recorded on a Bruker AC 300 MHZ FT-NMR spectrophotometer in $CDCl_3$ and chemical shift were recorded in parts per million downfield from TMS.

SYNTHESIS OF COMPOUND 1

A mixture of 12 gm of Bromo aniline and 10.64 gm of aldehyde dissolved in sufficient quantity of ethanol. The preparation is boiled at 300 volts 5-7 min in the microwave oven. After heating it is cooled in ice bath and water was added continuously till it becomes precipitate. The precipitate is filtered and dried at room temperature.

SYNTHESIS OF COMPOUND 2

A mixture of compound 1 is dissolved in 10 ml of DMF and Morpholine was dissolved in 5ml of DMF. The preparation is boiled at 300 volts 3-5 min in the microwave oven. After microwave heating it is cooled at room temperature and mixed with ice water and stirred continuously until the precipitate should be formed the precipitate is filtered and dried.

SYNTHSIS OF COMPOUND 3

A mixture of compound 2 and sufficient quantity of Hydrazine hydrate dissolved in 25 ml of ethanol. It was heated at microwave at 100 volts 3-5 mins. The mixture was cooled and poured in ice water by continuous stirring until precipitate should be formed it is filtered and dried. The product was recrystallized from ethanol.

4-(4-(4-methoxybenzylideneamino)phenyl)-N-phenylpiperazin-1-amine ($3a_1$) : IR (KBr/cm-1) 808 (s C-H), 1130 (C-H), 1314 m(C-N Stretching), 1361 (O-H) in plane bending, 1601(N-H in plan bending, 3313 s ((Ar), 2920 (CH) 589 (C-Br), 3480(–OH). ^{1}H NMR (DMSO-d6): δ = 6.39 -7.75 (m, 10H, Ar-H), 5.0 (1H, Aromatic – OH), 5.63 (s, 1H, CH), 4.13 (d, 2H, N-CH_2), 4.58 (d, 1H, CH-O), 2.27 – 3.57 (d, 8H, Morpholine) 3.73 (s, 3H, -OCH_3 , 2.0 (s, 1H, NH) ppm.

4-((4-(4-(phenylamino)piperazin-1-yl)phenylimino)methyl)phenol ($3a_2$) : IR (KBr/cm-1) 1725 (CO), 3345 (23.52) (NH), 1125 (C–O–C), 3095 (Ar), 2930 (CH), 589 (C-Br).^{1}H NMR (DMSO-d6): δ = 6.32 -7.15 (m, 12H, Ar-H), 5.0 (1H, Aromatic – OH), 5.63 (s, 1H, CH), 4.13 (d, 2H, N-CH_2), 4.58 (d, 1H, CH-O), 3.73 (s, 3H, -OCH_3 3.48 (d, 1H, CH-S), 2.27 (d, 3H, N-CH_3) 2.0 (s, 1H, NH) ppm.

4-(4-(4-(dimethylamino) benzylidene amino)phenyl)-N-phenylpiperazin-1-amine ($3a_3$): IR (KBr/cm-1) 3550 (OH), 1732 (CO), 3320 (NH), 3020 (Ar), 2928 (CH) 593 (C-Br), 1599 (C=C), 1120 (C–O–C). ^{1}H NMR (DMSO-d6): δ = 6.14 -7.74 (m, 11H, Ar-H), 5.0 (1H, Aromatic – OH), 5.63 (s, 1H, CH), 4.13 (d, 2H, N-CH_2), 4.58 (d, 1H, CH-O), 2.37 – 3.67 (d, 8H, Morpholine), 3.73 (s, 3H, -OCH_3 3.48 (d, 1H, CH-S), 2.85 (t, 2H, N –CH_3) ppm.

ANTI-OXIDANT ACTIVITY

In the present study, anti oxidant evaluation methods such as reducing power, phosphomolybdenum method were chosen to determine the antioxidant potential of the three compounds. The three compounds were dissolved in methanol to prepared 1000 µg/m solution. Solutions of different concentrations (50 µg/ml, 100µg/ml

and 150µg/ml) were prepared by serial dilution and the antioxidant activity evaluation was studied (Sonia M *et al*., 2008; A Hasanat *et al*., 2010; Hicham H *et al*., 2008).

SCHEME I

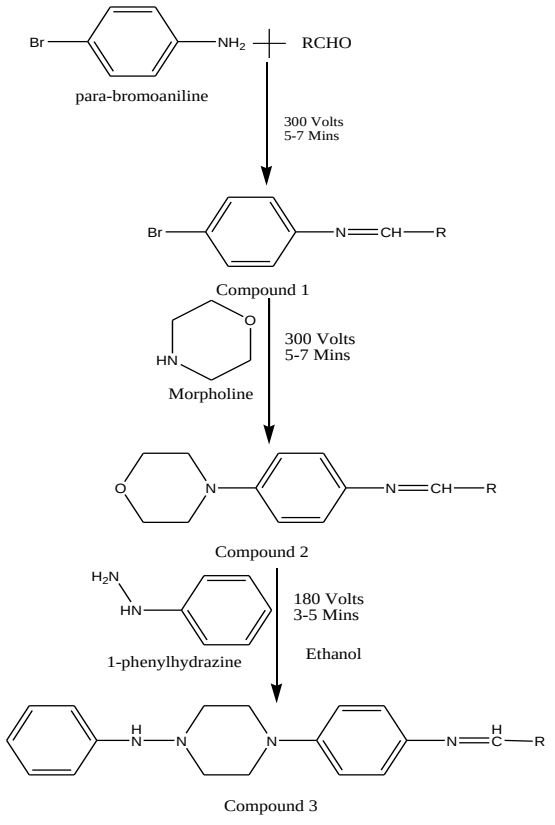
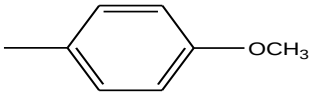
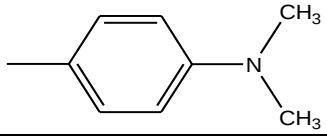
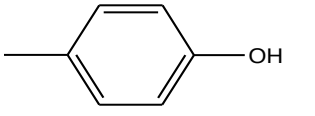


Table 1. Various Substitutions of the Synthesized Compounds $3a_1$- $3a_3$

S. No	Compound code	R
1	$3a_1$	
2	$3a_2$	
3	$3a_3$	

Determination of Total Antioxidant Capacity

The total antioxidant capacity (TAOC) was evaluated by the method of prieto et al. An aliquot of 0.1ml of sample solution (1mg/ml) was combined with ml of reagent solution (600mm H_2SO_4, 28mm sodium phosphate and 4mm ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°c for 90min. After the sample had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695nm against a blank. A typical blank solution contained 1ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same condition. The antioxidant capacity was expressed as the number of equivalents of BHT.

Reducing power assay

The reducing power was determined by taken the various concentration of sample that are mixed with 1ml of 200m mol/l sodium phosphate buffer (pH6.6) and 1% potassium ferric cyanide. The mixture was incubated at 50°c for 20 minutes. After 1ml of 10% trichloroacetic acid was added, the mixture was centrifuged at 2000rpm for 10 minutes. The upper layer solution (2.5ml) was mixed with 2.5ml of deionised water and 0.3ml of fresh ferric chloride (0.1%). The absorbance was measured at 700nm.

ANTIBACTERIAL ACTIVITY

Preparation

The ingredients were dissolved in water, and adjust the pH was to 7.2 to 7.4 by using dilute alkali/ dilute acid and autoclave at 121°C for 20 minutes. 30 – 35 ml of nutrient agar was transferred to a Petri dish.

Screening Of Anti Bacterial Activity

Bacterial strains of *Escherichia coli* (Gram –ve) and *Bacillus subtilis* (Gram +ve) were collected from Pharma lab, Pondicherry. 1000 µg/disc, 100 µg/disc & 10 µg/disc concentration of the test compounds are prepared & Dimethyl sulfoxide (DMSO) was used as vehicle. AMIKACIN (10 µg/disc) and KANAMYCIN (10 µg/ disc) was used as standard. Nutrient agar plates were prepared aseptically to get a thickness of 5 – 6 mm. The plates were allowed to solidify and inverted to prevent condensate falling on the agar surface. The plates were dried at 37°C just before inoculation. The standard inoculum is inoculated in the plates prepared earlier aseptically by dipping a sterile swab in the inoculum, removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of the liquid and finally streaking the swab all over the surface of 60 after each application. Finally press

the swab round the edge of the agar surface. The sterilized discs for the test drugs were placed in the Petri dishes aseptically. Incubate the Petri dish at 37°C for about 18 – 24 hrs, after placing them in the refrigerator for one hour to facilitate uniform diffusion. The average zone diameter of the plates were measured and recorded.

RESULTS AND DISCUSSION

Reducing power assay

Reducing power of piperazine and its three derivatives at different concentration (50 µM, 100 µM and 150 µM) was determined. In this assay, depending on the reducing power of antioxidant compounds the test solutions changes into various shades of green and blue colours. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reduction of ferric(Fe^{3+}) complex to the ferrous(Fe^{2+}) complex form can be monitored by measuring the formation of Perl's Prussian blue at 700 nm which occurs in the presence of reductants (antioxidant compounds). Reducing power of the tetrahydro Quinazoline, and its derivatives and standards (ascorbic acid) were determined using the potassium ferricyanide reduction method. The results are given in table 3.

Compound $3a_1$ possess good reducing power ability when compared to other compounds and the reducing power ability is close to the standard values (Ascorbic acid). The data presented here indicates that the reducing power observed in the study was in the following order compound $3a_1$ > BHT > $3a_3$ > $3a_2$ in various concentrations of samples. The data presented here indicate that the marked reducing power of compound $3a_1$ seems to be the result of their antioxidant activity.

Evaluation of antioxidant capacity by phosphomolybdenum method

The antioxidant activity for the synthesized compounds was evaluated by using phosphomolybdate method. It determines the total antioxidant capacity. This assay is based on the reduction of Mo (VI) to Mo (V) in presence of the antioxidant compounds and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH, which was measured at 695 nm. The antioxidant capacity of the compounds was determined for 50 µM, 100 µM and 150 µM concentrations. The antioxidant capacities of the compounds determined by phosphomolybdate method were expressed as µM of BHT equivalent/mg. From the values Compound $3a_1$ possess good antioxidant capacity whereas compound $3a_2$, $3a_3$ shows less antioxidant capacity. The antioxidant activity of the compounds were compared to standard (BHT) and found that compound $3a_1$ possess better phosphor molybdate activity than the standard. The results are given in table 4.

Table 2. Characterization data of synthesized compounds $3a_1$- $3a_3$

C. code	Molecular formula	M. W	M. P	Rf value	% yield	Λ max	Elemental analysis			
							C	N	O	H
$3a_1$	$C_{24}H_{26}N_4O$	386.5	272	0.5	69	210	74.6	14.5	4.14	6.78
$3a_2$	$C_{23}H_{24}N_4O$	372.5	270	0.4	68	210	74.2	15.0	4.30	6.49
$3a_3$	$C_{25}H_{29}N_5$	399.5	200	0.5	75	210	75.2	17.5	-	7.32

Table 3. Evaluation of antioxidant capacity by Phospho Molybdenum Method

S. No	Compound code	Concentration		
		50 µg/ml	100 µg/ml	150 µg/ml
1	$3a_1$	2.26 ± 0.01	2.21 ± 0.10	2.24 ± 0.01
2	$3a_2$	0.41 ± 0.16	0.83 ± 0.12	0.91 ± 0.14
3	$3a_3$	1.33 ± 0.04	1.30 ± 0.03	1.49 ± 0.08
4	BHT	2.26 ± 0.11	2.28 ± 0.03	1.55 ± 0.11

Table 4. Reducing power assay (Iron Reducing Activity)

S. No	Compound code	Concentration		
		50 µg/ml	100 µg/ml	150 µg/ml
1	$3a_1$	2.526 ± 0.29	3 ± 0	3 ± 0
2	$3a_2$	0.87 ± 0.18	1.00 ± 0.39	0.35 ± 0.07
3	$3a_3$	2.87 ± 0.22	2.92 ± 0.14	3.00 ± 0
4	Ascorbic acid	2.25 ± 0.11	2.24 ± 0.03	2.667 ± 0.04

Table 5. Antibacterial activity against *Escherichia Coli*

S. No	Compound code	Zone of inhibition in diameter			
		1000 µg /ml (A)	100 µg /ml (B)	10 µg /ml (C)	Standard (S)
1	Compound $3a_1$	16 mm	5 mm	-	23 mm
2	Compound $3a_2$	14mm	-	-	27mm
3	Compound $3a_3$	19mm	6 mm	-	30mm

Table 6. Antibacterial activity against *bacillus subtilis*

S. No	Compound code	Zone of inhibition in diameter			
		1000 µg /ml (A)	100 µg /ml (B)	10 µg /ml (C)	Standard (S)
1	Compound $3a_1$	15 mm	5 mm	-	22 mm
2	Compound $3a_2$	11 mm	-	-	23 mm
3	Compound $3a_3$	9 mm	-	-	11 mm

(--) indicate no zone of inhibition

OBSERVATION

Micro organism – *Bacilus subtilis* (Gram +ve)

Fig 1. Compound $3a_1$

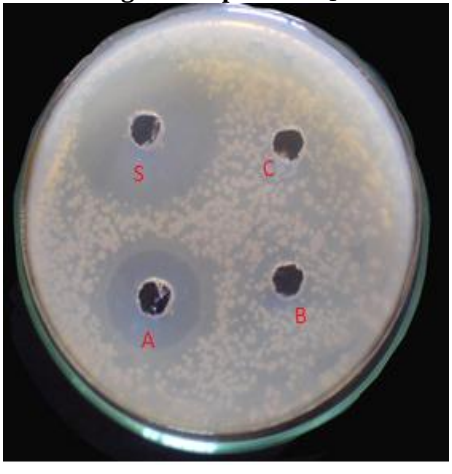


Fig 2. Compound $3a_2$

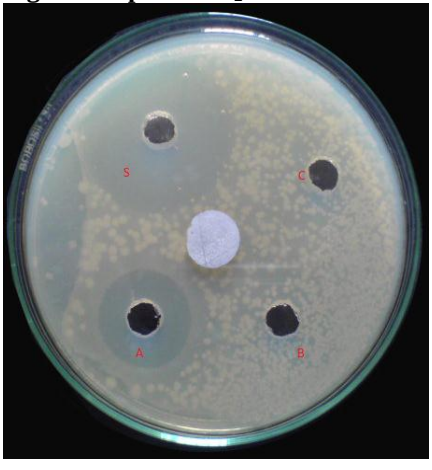


Figure: 3 Compound $3a_3$

Micro organism – Escherichia coli (Gram -ve)

Fig 4. Compound $3a_1$

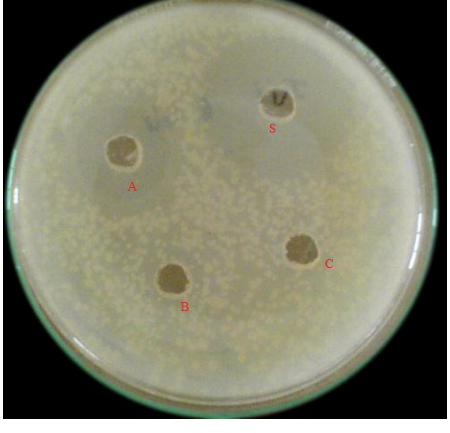


Fig 5. Compound $3a_2$

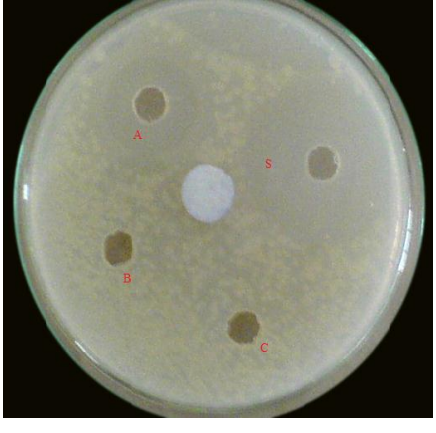


Fig 6. Compound $3a_3$

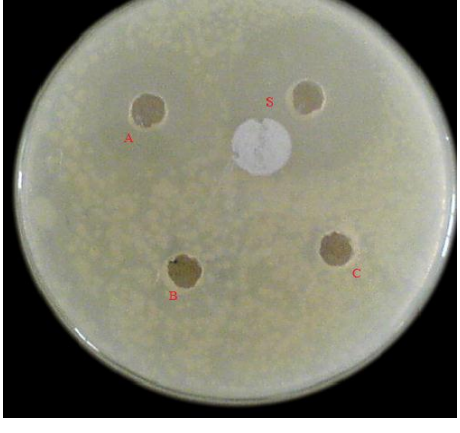


Fig 7. Evaluation of Antioxidant Capacity by Phospho Molybdenum

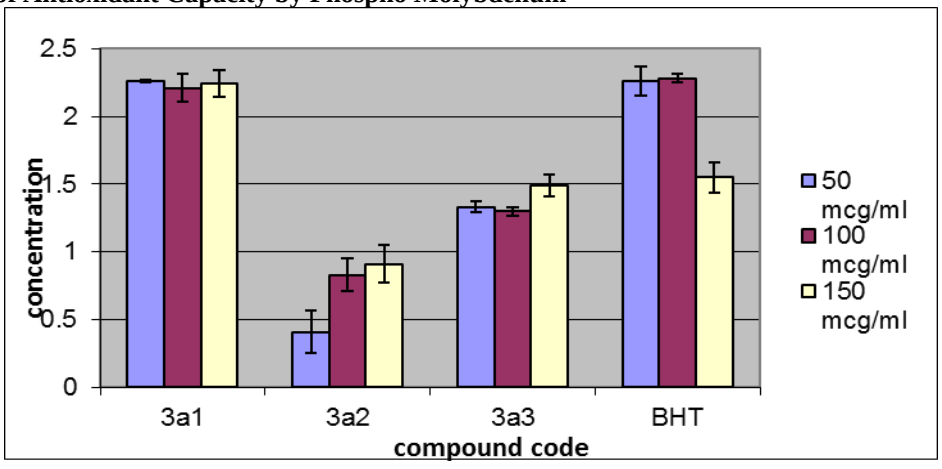
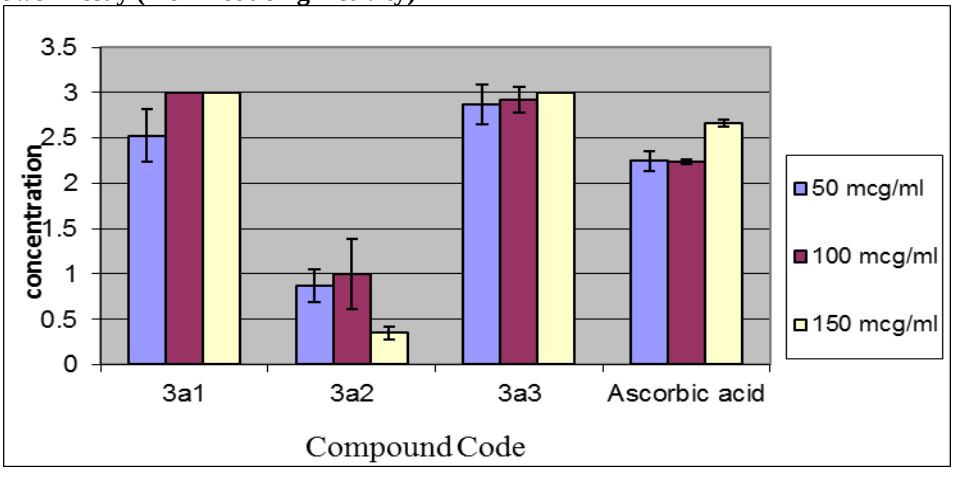


Fig 8. Reducing Power Assay (Iron Reducing Activity)



ANTIBACTERIAL ACTIVITY

Activity against Gram Positive organisms

Compound $3a_1$, $3a_2$ and $3a_3$ at 1000 mg/ml was found to be great and good activity against *Bacilus subtilis*. The results are given in table 6.

Compound $3a_1$ at 100 mg/ml was found to have minimum activity against *Bacilus subtilis*.

Activity against Gram negative organisms

Compound $3a_1$, $3a_2$ and $3a_3$ at 1000 mg/ml was found to be great and good activity against *Escherichia coli*. The results are given in table 5.

Compound $3a_1$ and $3a_3$ at 100 mg/ml was found to have minimum activity against *Escherichia coli.*

CONCLUSION

The three piperazine derivatives were synthesized by using different aldehydes. The synthesized compounds were evaluated for physical characterization like, molecular weight, melting point and R_f value. Piperazine and its derivative analogues were successfully synthesized from the known methods and the antioxidant activity and antibacterial activity for the synthesized compounds was evaluated. The results of the present experiment shows that compound $3a_1$ showed more promising antioxidant activity against reducing power assay, phosphomolybdate method. The most active compounds like piperazine and its derivative have shown better antioxidant activity compared to the standards like BHA and ascorbic acid respectively. None of the test compounds could exhibit significant antibacterial activity comparable to that of the standard Amikalin and Kanamyin. Synthesized compound was shown to exhibit good antibacterial activity against *Escherichia coli* and *Bacillus subtilis.* Finally, it could be conclused from the above results that the piperazine derivative has good Anti-bacterial activity. This will

provides the theoretical information for the medicinal development, and supplies some *in vitro* methods for quick-optimization of drugs, which can be useful for the potential source of synthetic antioxidant. We suggest that to synthesize more compounds in this scheme and pharmacological evaluation, further studies required to prove the pharmacological activity of the same.

REFERENCES

Hasanat A, Zulfiker MD, Farhana AR, Md. Mahbubur RM. Obayed U, Kaiser H, Md. Mahbubur RK, Sohel Rana MD. Antidiabetic and antioxidant effect of scoparia dulcis in alloxan induced albino mice, *International Journal of Pharma Tech Research Coden (USA): IJPRIF*, 2(4), 2010, 2527-2534.

Hicham H and Souliman A. Pharmacognosy review article spectrophotometric methods for determination of plant poly phenols content and their antioxidant activity assessment, an over view, *Pharmacognosy Reviews*, 2(3), 2008, 20-23.

Raj.K.Bansal. Heterocyclic chemistry. 5th edition, 2005, 528 - 535.

Roger Walker. Clinical Pharmacy and Therapeutics, 3rd edition, 2003, 584.

Sonia M, Mohmad D. *In-vitro* antioxidant activities of aloe Vera leaf skin extracts, *Jour De Al Societe Chimique De Tunisine*, 10, 2008, 101-109.

Surendra NP. *Text Book of Medicinal Chemistry*, 3th edition, 2003.

Tripathi KD. Essentials of Medical Pharmacology, 5th edition, 2003.

Vijaykumar H, Gnanendra CR, Nagaraja Naik and Channe Gowda D. Invitro antioxidant activity of dibenz (b,f) azepine and it analogues, *E-J Chem*, 5(s2), 2008, 1123-1132.