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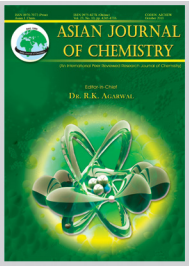

Syntheses, Spectral Characterization and Biological Elucidation of Some Mannich Bases

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Some biologically potent Mannich bases have been prepared from 2-butanone using aromatic aldehydes and aromatic amine in ethanol under acidic conditions. The synthesized Mannich bases were purified through standard chromatographic techniques. The geometry of the novel Mannich bases were established by various spectroscopic techniques like ^{1}H NMR, mass and IR spectroscopy. Finally these bases were exposed to various microorganisms in order to establish their bioactivity.

Key Words: Syntheses, Spectra, Biological properties, Mannich bases.

INTRODUCTION

A Mannich base is an end product in Mannich reaction and may generally be defined as *N,N*-dialkyl amino methyl derivative of active hydrogen compound. The Mannich reaction (Carl Mannich 1877-1947)[1] is a three-component reaction, which involves the condensation of a compound capable of supplying one or more active hydrogen atoms with an aldehyde (usually formaldehyde) and an N-H derivative (ammonia, any primary or secondary amine or amide)[2] in the presence of an acid to give β-amino carbonyl compounds[3,4]. The essential feature of Mannich reaction is the replacement of active hydrogen atom of an organic molecule by an amino alkyl or substituted amino alkyl group. The Mannich reaction or more general α-amino alkylation of a compound having an active hydrogen atom is an important preparative C-C bond forming reaction in organic synthesis and one of the most widely utilized chemical transformations for constructing β-amino carbonyl compounds. β-Amino carbonyl compounds are important synthetic intermediates for various pharmaceuticals and natural products[5]. The amine of the reaction may preferably be introduced as its salt (amine hydrochloride Mannich bases and their derivatives exhibit a large number of interesting applications in organic synthesis. Among these the preparation of pharmaceuticals and natural products (peptides, nucleotides, antibiotics and alkaloids) is of capital importance[6,7]. In addition Mannich bases have been used for the synthesis of organometallics and agrochemicals such as plant growth regulators[8], paint and polymer chemistry[9], surface active compounds, as precursor to optically active amino acids, curring, catalyst, cross linking agents[10], dispersants in lubricating oils[11] and product for motor fuels[12]. Mannich bases show local anesthetics property[13] various drugs obtain from Mannich reaction have proved more effective and less toxic than their parent antibiotics[14].

It also provides a convenient access to many useful synthetic building blocks because amino group can be easily converted into a variety of other functionalities[15,16]. This reaction also offers a convenient method for production of the basic amino alkyl chain, which alters the biological profile and physicochemical characteristics[17].

In spite of its synthetic utility, the Mannich reaction generally involves some limitations *i.e.* longer reaction time, expensive chemicals, large amount of catalyst *etc*. The major drawback is that some times reaction may not stop at the initial stage but it proceeds further which automatically results in the formation of low yield of the desired product. Also the product separation becomes costly, difficult and time taking.

Previously a number of biologically active molecules are known by our group[18-24], as an extension of our curiosity for the search of bioactive molecules, we are now interested in the development of the chemistry of some novel mannich bases and also intended to screen them for wide variety of bacteria and fungi.

EXPERIMENTAL

All the glassware used were cleaned with chromic mixture, detergent and finally with distilled water and then dried in

oven at 110 ºC. All the solvents used in the experiment were purified and dried according to standard procedures[25]. All the chemicals used were of analytical grade and used as such without further purification.

TLC was performed on precoated silica gel (E-Merck) 60F-254 analytical plates (0.25 mm). The solvent system used was *n*-Hexane/ethyl acetate (1:9). Melting points were determined on Gallen kamp digital (England) melting point apparatus. The observed melting points are in ºC and are uncorrected. FTIR spectra were recorded on Bruker FTIR Tensor 27 spectrophotometer; NMR spectra were recorded on Bruker 300 MHz FTNMR spectrophotometer. Mass spectra were recorded on GC- MS model MAT 312.

Synthesis of Mannich bases: 10 mmol of corresponding aromatic amine were dissolved in a 15 mL of ethanol in a 100 mL 2-neck round bottom flask equipped with a reflux condenser protected by a calcium chloride drying tube and a quick fit thermometer, then 10 mmol of 2-butanone and 10 mmol of an aromatic aldehyde were dissolved with constant stirring, to this reaction mixture 0.2 to 0.4 mL HCl were added with cooling on an ice bath. Yellow colour solid separates just after the addition of HCl. The mixture was stirred for 1-2 h at room temperature. The reaction mixture was refluxed on a water bath at 90-95 ºC for 3-4 h, the reaction was examined by TLC with time to time till completion. The excess of solvent was removed under reduced pressure; the product was washed with water and 95 % ethanol respectively. The product was purified by column chromatography using hexane-ethyl acetate (3:7) system.

1-(3-Methoxyphenyl)-1-(4-methylphenyl amino)-3-pentanone: This compound was obtained as white solid, 75 % yield, m.p., IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O), 3365 (N-H) 3040 (C-H-Ar), 1590 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.5 (q, 2H, CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.6 (t, 1H, CH_2-CH), δ 2.5 (s, 3H, CH_3), δ 6.7-7.0 (m, 8H, Aro), δ 3.8 (s, 1H, NH); *m/z*. 29 (14), 57 (30), 91 (65), 226 (100), 297 (M^+, 2).

1-(3,4-Dimethoxyphenyl-1-(4-methylphenyl amino)-3-pentanone: This compound was obtained as white solid, 75 % yield, m.p. IR (KBr, ν_{max}, cm^{-1}): 1697 (C=O), 3400 (N-H) 3090 (C-H-Ar), 1601 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.4 (q, 2H, CH_3-CH_2), δ 3.6 (s, 6H, 2-OCH_3), δ 2.9 (d, 2H,CH_2-CH), δ 4.7 (t, 1H, CH_2-CH), δ 2.2 (s, 3H, CH_3), δ 6.2-6.9 (m, 7H, Aro), δ 4.2 (s, 1H, NH); *m/z*. 29 (14), 57 (30), 91 (80), 256 (100), 327 (M^+, 2).

1-(3,4,5-Trimethoxyphenyl)-1-(4-methylphenyl-amino)-3-pentanone: This compound was obtained as white solid, 73 % yield, m.p., IR (KBr, ν_{max}, cm^{-1}): 1711 (C=O), 3340 (N-H) 3060 (C-H-Ar), 1610 (C=C-Ar). ^{1}H NMR δ 0.9 (t,3H, CH_3-CH_2), δ 2.4 (q, 2H, CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.8 (t, 1H, CH_2-CH), δ 2.2 (s, 3H, CH_3), δ 6.0-6.7 (m, 6H, Aro), δ 3.9 (s, 1H, NH), δ 3.6 (s, 9H, 3-OCH_3; *m/z*. 29 (14), 57 (30), 91 (50), 286 (100), 357 (M^+, 4).

1-(4-Hydroxy, 3-methoxyphenyl)-1-(4-methylphenyl-amino)-3-pentanone: This compound was obtained as white solid, 70 % yield, m.p.; IR (KBr, ν_{max}, cm^{-1}): 1700 (C=O), 3361 (N-H) 3083 (C-H-Ar), 1490 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.3 (q, 2H, CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.6 (t, 1H, CH_2-CH), δ 2.5 (s, 3H, CH_3), δ 6.3-6.9 (m, 7H, Aro), δ 3.8 (s, 1H, NH), δ 5.3, (s, 1H, OH); *m/z*. 29 (14), 57 (30), 91 (65), 242 (100), 313 (M^+, 2).

1-(4-Flourophenyl)-1-(4-methylphenylamino)-3-pentanone: This compound was obtained as white solid, 69 % yield, m.p.; IR (KBr, ν_{max}, cm^{-1}): 1699 (C=O), 3390 (N-H), 3000 (C-H-Ar), 1570 (C=C-Aro); ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.4 (q, 2H, CH_3-CH_2) δ 2.9 (d, 2H, CH_2-CH), δ 4.6 (t, 1H, CH_2-CH), δ 6.4-7.0 (m, 8H, Aro), δ 2.3 (s, 3H, -CH_3), δ 3.8 (s, 1H, NH). *m/z*. 29 (14), 57 (30), 91 (65), 214 (100), 285 (M^+, 3).

1-(4-Chlorophenyl)-1-(4-methylphenylamino)-3-pentanone: This compound was obtained as white solid, 70 % yield, m.p.; IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O), 3364 (N-H), 3045 (C-H-Ar), 1580 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.4 (q, 2H,CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.7 (t, 1H, CH_2-CH), δ 6.3-7.2 (m, 8H, -Aro), δ 2.4 (s, 3H, CH_3), δ 3.9 (s, 1H, NH). *m/z*. 29 (14), 57 (30), 91 (80), 230 (100), 301 (M^+, 6).

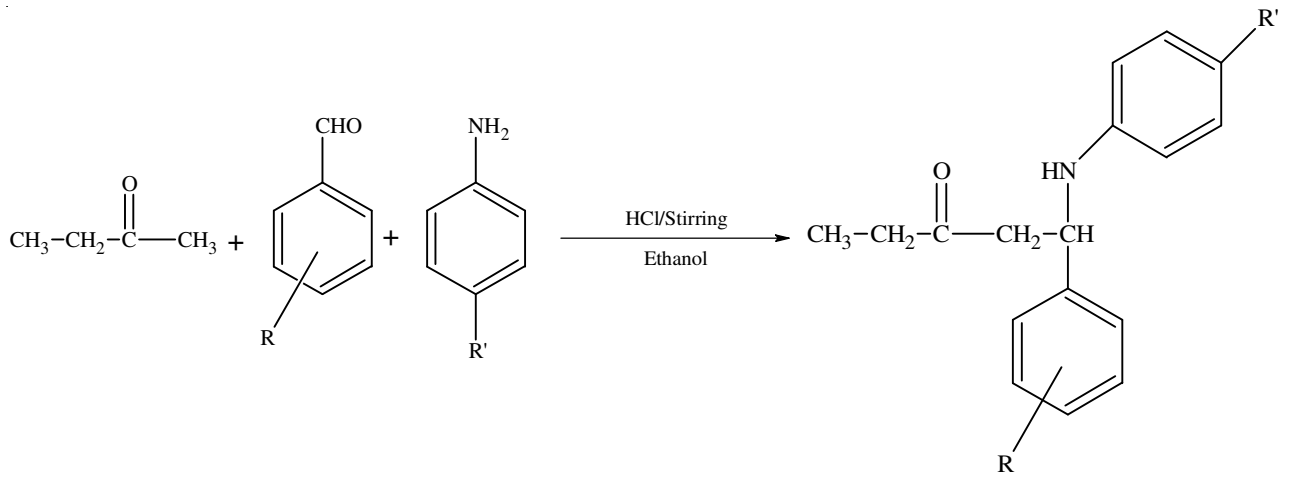
1-(4-Bromoyphenyl)-1-(4-methylphenylamino)-3-pentanone: This compound was obtained as white solid, 78 % yield, m.p.; IR (KBr, ν_{max}, cm^{-1}): 1711 (C=O), 3375 (N-H), 3060 (C-H-Ar), 1610 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.4 (q, 2H, CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.8 (t, 1H, CH_2-CH), δ 6.2-7.3 (m, 8H, -Aro), δ 2.3 (s, 3Hs, 3H, CH_3), δ 3.9 (s, 1H, NH). *m/z*. 29 (14), 57 (30), 91 (95), 274 (100), 345 (M^+, 5).

1-(4-Iodophenyl)-1-(4-methylphenylamino)-3-pentanone: This compound was obtained as white solid, 80 % yield, m.p.: IR (KBr, ν_{max}, cm^{-1}): 1710 (C=O), 3390 (N-H), 3040 (C-H-Ar), 1600 (C=C-Ar). ^{1}H NMR δ 0.9 (t, 3H, CH_3-CH_2), δ 2.4 (q, 2H, CH_3-CH_2), δ 2.9 (d, 2H, CH_2-CH), δ 4.8 (t, ^{1}H, CH_2-CH), δ 6.4-7.5 (m, 8H, -Aro), δ 2.3 (s, 3H, 3H, CH_3), δ 4.2 (s, 1H, NH). *m/z*. 29 (14), 57 (30), 91 (85), 322 (100), 393 (M^+, 5).

Biological studies: All the bases were tested against various organisms for the following activities.

Brine shrimp lethality bioassy: Artificial sea water was prepared by dissolving 3.8 g sea salt per liter of double distilled water and filtered. Sea water was placed in a small tank and shrimp eggs (Artemia Salina) (1 mg) were added to the large compartment of the tank, which was darkened by covering it with aluminum foil. The illuminated compartment attracted shrimps larvae through perforation in the dam. It was allowed to stand for 24 h at 25 ºC for the shrimps to hatch and mature. Different concentrations (150, 100, 150 µg/mL) of the test samples were prepared in DMSO. Prepared 3 replicates for each concentration making a total of 24 vials. After 2 days when shrimp larvae matured added 5 mL sea water to each vial and added 12 shrimps per vial, allowed standing for 24 h under illumination. After 24 h counted and recorded the number of surviving shrimps. Analyzed data with a Finney computer program to determine the LD_{50} values[26].

Antibacterial activity: The antibacterial activity was determined by using the agar well diffusion method[27]. The wells were dug in the media with a sterile borer and 8 h old bacterial inoculums containing *ca.* 10^4-10^6 colony-forming units (CFU)/mL was spread on the surface of the nutrient agar using a sterile cotton swab. The recommended concentration of the test sample (2 mg/mL in DMSO) was introduced in the



Scheme-I

Where, R = 3-OCH_3; 3,4-OCH_3; 3,4,5 -OCH_3; 3-OCH_3, 4-OH; 4-F; 4-Cl; 4-Br; 4-IR' = 4-CH_3

respective wells. Others wells containing DMSO and the reference antibacterial drug served as negative and positive controls, respectively. The plates were incubated immediately at 37 ºC for 20 h. The activity was determined by using the diameter of the inhibition zone (mm) showing complete inhibition. Growth inhibition was calculated with reference to the positive control.

Antifungal activity: The dilution plate method[28] was used for isolation of fungi. Selected and isolated fungi were maintained on potato dextrose agar plates at 4 ºC for further experimental work. The antifungal activities of the ligands, mixed ligand complexes, metal nitrates, fungicides (bavistin and emcarb) and the control DMSO (dimethylsulfoxide) were screened using the plate poison technique[29]. Seven day-old cultures of *Aspergillus niger*, *Fusarium oxysporum* and *Aspergillus flavus* were used as test organisms. A stock solution of 500 g/mL was made by dissolving 50 mg of each compound in dimethyl sulphoxide (100 mL). The sterilized medium with the added stock solution was poured into 90 mm sterile petri plates and allowed to solidify. They were inoculated with a 5 mm actively growing mycelial disc and incubated at 27 ºC for 72 h. After 72 h of inoculation, the percent reduction in the radial growth diameter over the control was calculated. The growth was compared with dimethyl sulfoxide as the control.

RESULTS AND DISCUSSION

The Mannich bases have been synthesized by reacting 2-butanone with aromatic aldehydes and primary aromatic amine under acidic conditions using ethanol as solvent **Scheme-I**. The physico-chemical charcteristics of the synthesized Manich compound are given in Table-1.

The FTIR spectrum of Mannich bases showed strong absorption in the region of N-H and C=O ranging from 3400-3340 and 1740-1697 respectively. The ^1H NMR spectrum showed two expected signals at 0.9 ppm (t) and 2.9 ppm (t), which are characteristics of methyl and methylene groups respectively present in the structure. The signals of aromatic protons came at the range of 6.7-7.4 ppm (m). The mass spectrum showed two large peaks at *m/z*. 29 and 57, which indicate the formation of CH_3CH_2 and $CH_2CH_2CO^+$ respectively.

TABLE-1
SYNTHESIS OF MANNICH BASES

Mannich base	R	R'	m.f.	m.w.	m.p.
1	3- OCH_3	4-CH_3	$C_{19}H_{23}NO_2$	297	111
2	3, 4-OCH_3	4-CH_3	$C_{20}H_{25}NO_3$	327	92
3	3, 4, 5-OCH_3	4-CH_3	$C_{21}H_{27}NO_4$	357	121
4	3-OCH_3, 4-OH	4-CH_3	$C_{19}H_{23}NO_3$	313	103
5	4-F	4-CH_3	$C_{18}H_{20}FNO$	285	96
6	4-Cl	4-CH_3	$C_{18}H_{20}$ Cl NO	301	88
7	4-Br	4-CH_3	$C_{18}H_{20}BrNO$	345	90
8	4-I	4-CH_3	$C_{18}H_{20}$ I NO	393	115

Biological activities

Brine shrimp bio-assay: Bioactive compounds are often toxic to shrimp larvae, hence *in vivo* lethality to shrimp larvae can be used as simple and convenient preliminary monitor for new bioactive synthetic products[30,31]. Brine shrimp napulii have been used previously in a number of bioassay system[32].

This is rapid, inexpensive, in-house general bioassay that may serve as an intermediate test before further *in vivo* animal experiments on large scale.

All the synthesized Mannich bases were investigated for their cytotoxic property by brine shrimp lethality bioassay. Most of them have shown significant activity against brine shrimp nauplii. The LD_{50} values of the synthesized bases are presented in Table-2.

TABLE-2
BRINE SHRIMP BIOASSAY OF MANNICH BASES

Mannich base	% age of mortality at doses			LD_{50}
	50 µg/mL	100 µg/mL	150 µg/mL	
1	4	9	11	45.02
2	75	80	98	25.52
3	8	7	5	51.04
4	85	15	20	0.053
5	34	52	75	111.20
6	37	95	24	1.26
7	4	8	11	45.12
8	10	20	60	61.77

The Mannich base **4** was found to be highly active with LD_{50} value of 0.053 µ/mL. The compounds **1**, **2** and **6** also showed good activity with LD_{50} of 45.02, 25.52 and 1.26

TABLE-3
ANTI BACTERIAL ACTIVITY RESULTS OF MANNICH BASES

Bacteria	High concentration samples								*Standard Drug
	1	2	3	4	5	6	7	8	
Bacillus subtilIs	++	++	++	+++	+	n.a.	+	n.c.	++++
Staphylococcus aureus	+++	+	+	++	+	++	n.c.	n.a.	++++
Escherichia coli	+	+++	+	+++	+	+	n.a.	+	++++
Schigella flexenari	n.a.	++	+	+++	++	+++	+	+	++++
Pseudomonas aeruginosa	++	++	++	++	+	++	n.a.	+	++++
Salmonella typih	++	++	+	+++	+	+	+	++	++++

*Impinium; ++++ highly active, +++ good activity, ++ moderate activity, significant activity, n.a.: no activity, n.c.: not checked

μ/mL respectively. While rest of the synthesized Schiff bases also exhibit significant activity. The activity order is as 4 < 6 < 2< 1 < 7 < 3 < 8 < 5.

Antibacterial Studies: The resulting synthesized Mannich and Schiff bases were checked against various microorganisms such as *Salmonella typhi*, *Schigella flexenari*, *Escherichia coli, Staphylococcus aureus* and *Bacillus subtills* in order to establish their bioactivities. In order to compare the results obtained the Impinem is used as a standard drug. The result obtained shows that synthesized bases have significant activity but lower than the standard drug. The result indicate that the base 1, 2 and 4 showed promising activity because it is proposed that oxygen of methoxy group may bind with bacteria and hence play an important role in enhancing the activity. Among these three, compound 4 has greater activity because of hydroxyl group, which enhances the biological activity[33]. Where as base 6 is found to be of moderate activity. However Schiff base 3, 5, 7 and 8 showed somewhat less inhibition than others. The antibacterial activity results of these bases are presented in Table-3.

Antifungal activity: The four different fungi *C. gloeosporiodes, A. brassicicola, A. brassicae, C. capsici* and *H. graminium* were used in order to establish antifungal activity of the complexes.

The results of the antifungal activity are given in Table-2, the results obtained showed that the chloro Mannich basis are more toxic than the others against the same fungi. The increase in the antifungal activity of the chloro derivatives may be due to the effect of the chloro ion on the normal cell processes. A possible mode for the toxicity increase may be considered in light of Tweede's chelation theory[34]. Although there is a sufficient increase in the fungicidal activity of the chloro mannich bases as compared to other derivatives and the control (dimethyl sulfoxide), they cannot accomplish the value of the conventional fungicides (bavistin and emcarb). However, differences in antifungal activity may result from differences in chemical structures, which may affect their interaction with receptors involved in the biological activity.

REFERENCES

1. C. Mannich and W. Krosche, *Arch. Pharm*., **250**, 647 (1912).
2. H. Hellmann and G. Oftiz, *Newer Methods Prep. Org. Chem.*, **2**, 277 (1963).
3. M. Tramontini and L. Angiolini, *Tetrahedron*, **46**, 1791 (1990).
4. O.P. Agrawal, Organic Reactions and Reagents, Goel Publishing House India, edn. 38, p-804 (2004).
5. F.A Davis, Y. Zhang and G. Anilkumar, *J. Org. Chem.*, **68**, 8061 (2003).
6. R.O.C. Norman, Principles of Organic Synthesis, Chapman and Hall, London, edn. 2, pp. 734-740 (1978).
7. R.J. Robenson, *J. Chem. Soc.*, **111**, 762 (1917).
8. S.K. Sridhar, M. Saravanan and A. Ramesh, *Eur. J. Med. Chem.*, **36**, 615 (2001).
9. S. Joshi, N. Khosla, D. Khare and R. Sharda, *Bioorg. Med. Chem. Lett.*, **15**, 221 (2005).
10. A. Chipeleme, J. Gut, P.J. Rosenthal and K. Chibale, *Bioorg. Med. Chem.*, **15**, 273 (2007).
11. K.V. Sujith, N. Jyothi, P.S. Rao and K. Balakrishna, *Eur. J. Med. Chem.*, **44**, 3697 (2009).
12. M.A. Ali and M. Shaharyar, *Bioorg. Med. Chem. Lett.*, **17**, 3314 (2007).
13. H. Suleyman, H. Inci Gul and M. Asoglu, *Pharmacol. Res.*, **47**, 471 (2003).
14. W. Malinka, P.S. Wittek, B. Filipek, J. Sapa, A. Jezierska and A. Koll, *IL Farmaco*, **60**, 961 (2005).
15. M. Tramontini and L. Angiolini, *Tetrahedron*, **46**, 1791 (1990).
16. A. Pochini, G. Puglia and R. Ungaro, *Synthesis*, 906 (1983).
17. S. Joshi and N. Khosla, *Indian Drugs*, **31**, 548 (1994).
18. W. Rehman, A. Badshah, S. Khan, L.T.A. Tuyet, *Eur. J. Med. Chem.*, **44**, 3981 (2009).
19. W. Rehman, M.K. Baloch and A. Badshah, *Eur. J. Med. Chem.*, **43**, 2380 (2008).
20. W. Rehman, F. Saman and I. Ahmad, *Russ. J. Coord. Chem.*, **34**, 678 (2008).
21. W. Rehman, B. Muhammad, F. Ali and S. Atif, *Chin. J. Struct. Chem.,* **27**, 846 (2008).
22. W. Rehman, A. Badshah, S. Khan and I. Ahmad, *Chin. J. Struct. Chem.*, **28**, 419 (2009).
23. W. Rehman, S. Rehman, B. Muhammad, S.T. Shah, I. Tauseef and C.Y. Guo, *Polish J. Chem.*, **83**, 2043 (2009).
24. W. Rehman, M.K. Baloch and A. Badshah, *J. Braz. Chem. Soc.*, **16**, 827 (2005).
25. D.D. Perrin and W.L.F. Armengo, Purification of Laboratory Chemicals, Pergamon, Oxford (1988).
26. Finney, Probit Analysis, Cambridge University Press, Cambridge, edn. 3 (1979).
27. A. Rahman, M.I. Choudhary and W.J. Thomsen, Bioassay Techniques for Drug Development, Harwood Academic Publishers, The Netherlands, p. 16 (2001).
28. B.G. Tweedy, Elemental Sulfur, in ed.: D.C. Torgeson, Fungicides. An Advanced Treatise, Chemistry and Physiology, Academic Press, London, UK, Vol. **2**, p. 119 (1969).
29. J.B. Chauhan, R.B. Subramanian and P.K. Sanyal, *Indian J. Environ. Toxicol.*, **12**, 22 (2002).
30. G. Persoone, Proceedings of the International Symposium on Brine Shrimps, Artemia Salina, Universal Press, Belgium, Vol. 1-3 (1980).
31. K. Shoaib, W. Rehman, B. Muhammad and S. Ali, *J. Proteomics Bioinform.*, S8 (2012); DOI: http://dx.doi.org/10.4172/jpb.S8-001.
32. B. Furniss, A.J. Hannaford, P.W.G. Smith and A.R. Tatchell, Vogel's Text Book of Practical Organic Chemistry 5th Ed, ELBS Longman group. U.K (1989).
33. Atta-ur-Rahman, Studies in Natural Product Chemistry, Netherland Elsevier Science Publisher, B.V., Vol. 9, p. 383 (1991).
34. B.G. Tweedy, *Phytopathology*, **55**, 910 (1964).