Executive Summary

A MINOR RESEARCH PROJECT CARRIED OUT UNDER THE FINANCIAL ASSISTANCE OF UNIVERSITY GRANTS COMMISSION, WRO, PUNE.

"CONVENTIONAL AND MICROWAVE SYNTHESIS OF SOME SCHIFF BASES AND THEIR ANTIBACTERIAL ACTIVITY"

Submitted by:

Prof. S. B. Salve,
Principal Investigator

Dr. Hemant A. Mahajan,
Co-Investigator
at
Smt. G. G. Khadse Science and Arts College, Muktainagar
Dist. - Jalgaon, (MS) Pin. 425306

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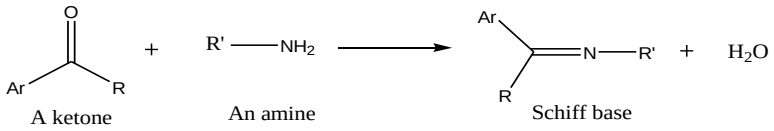
Introduction:

A compound formed by the reaction between an aromatic amine and an aldehyde or ketone is known as Schiff base or Ketimine. Schiff bases were first discovered by Hugo Schiff[1] and hence they are referred as ketimine. In general a ketimine can be prepared as follows.



A number of reviews of the ketimines or ketimine have been published[2]. The kinetics of formation of ketimines in aqueous solution have been extensively studied[3-4]. This reaction proceeds by a two-step mechanism involving a carbinolamine intermediate. Some ketimines or Schiff bases have been studied for electrochemistry[4-5], Fluorescence[6] and Luminescence[7].

Schiff base or ketimines are the very versatile and useful as Model s for such important studies. So herein we studied ketimines formed from 5-Chloro-2-hydroxy-4-methyl-acetophenone and substituted anilines.

Why microwave has been studied ?

The conventional method requires more tedious apparatus and it takes more time for the results. Hence some researchers have thought of reducing the time and improvising the results. Which was the motive to develop newer methods and in this process microwave method emerged out.

In conventional synthesis of Ketimine, it commonly meets the problem of removing solvent from the reaction mixture or liquid extraction especially in the case of aprotic dipolar solvent with high boiling point, or product isolation through liquid-liquid extraction.

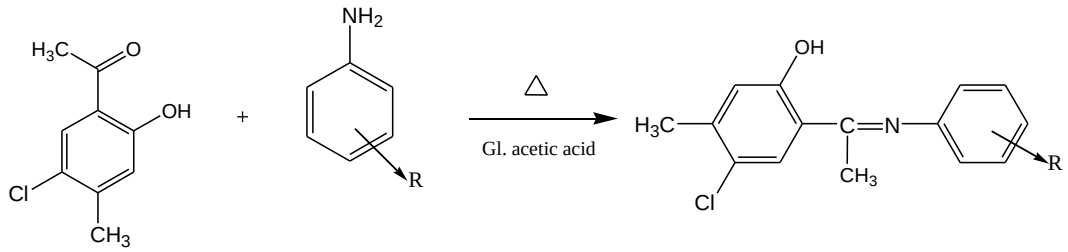
Microwave-assisted reactions have been intensively investigated as mentioned in previous study[8-10]. Microwave-assisted technique has been popularly used in organic synthesis. The organic synthesis mediated by microwave irradiation performs several advantages such as higher atom economy, environmental friendship, reducing the hazards[8-11]. Literature shows that many researchers have used this method to synthesize the Ketimine or Aldimine using aromatic aldehyde and varied anilines and hetero-aromatic amines were condensed by microwave promoted synthesis[12]. N-Substituted benzylidene aniline derivatives were synthesized by microwave and their antifungal activity are studied[13]. Recently, we have reported microwave synthesis of Schiff bases from Benzaldehyde[14] o-Hydroxy-acetophenone[15], Benzophenone[16]. Schiff base have been investigated by FTIR[17] and ^{1}H NMR[18] spectra and Voltammetry[19]. Many type of reaction like metal complex formation oxidation, addition, hydrolysis, reduction and substitution have been studied with ketimines.

Brief objective of the project:

1. The literature survey pertaining to study the synthesis of ketimines by using Aromatic ketone with seven different aromatic amines.
2. To synthesize and characterize ketimines from the Aromatic ketone with seven different aromatic amines viz. Aniline, 4-Amino-phenol, 2-Amino-thiophenol, 4-Amino-thiophenol, 4-Chloro-2-nitro-aniline, 2-Amino-benzonitrile and 4-Amino-benzonitrile.
3. To study the purification and characterization of the synthesized ketimines by using elemental analysis, colour, physical constant and TLC with varied spectroscopic methods such as UV-Vis, FTIR and HPLC.
4. To synthesize ketimine-complexes and to study their elemental analysis, physical constant, TLC and colour with varied spectroscopic methods such as UV-Vis, FTIR and HPLC.
5. To determine the Antibacterial activities of Ketimines or Schiff base derivatives.
6. To conduct the microwave synthesis of Ketimine derivatives of above pair of ketone and seven amines and compare their yields.

Scheme of Work:

Scheme-1



1-(5-Chloro-2-hydroxy-4--methyl-phenyl)-ethanone

Substituted aniline

4-Chloro-5-methyl-2-(1-substituted--phenylimino-ethyl)-phenol

R- = -H (S-I); -4-OH (S-II); -2-SH (S-III);
-4-SH (S-IV); -4Cl-2-NO_2-(S-V);
-2-CN(S-VI) and -4-CN(S-VII).

Origin of the Research Problem:

Schiff base were first synthesized by H. Schiff[1] and they are useful as intermediates[2] in many reactions. It has varied applications in the field of synthesis[2], medicine[20], catalysis[21-22], drug-intermediates[23a,b], dyes[24] and analysis[25]. Many types of reaction like complexation[26], reduction[27-28] and oxidations[29] have been studied with ketimines. The ketimines also plays important role in many biochemical reactions[30-31]. Ketimines also exhibits antibacterial[32], antifungal[33], drug-bioactivity[34] and anticancer[35] activity.

Schiff base have been synthesized by various methods. Looking to this we propose to study the synthesis of the same schiff bases by Microwave method also. The comparision of their purity and the yields will be commented.

Methodology:

I. Preparation of Ketimines from 5-Chloro-4-methyl-2-hydroxy-acetophenone by

i) Conventional (Method-A): (for details see our publication Der. Chemica Sinica., commun., Dec. 2015**).**

ii) Microwave (Method-B): (for details see our publication Der. Pharma. Chemica., commun., Dec. 2015**).**

II. Analysis of Ketimines:

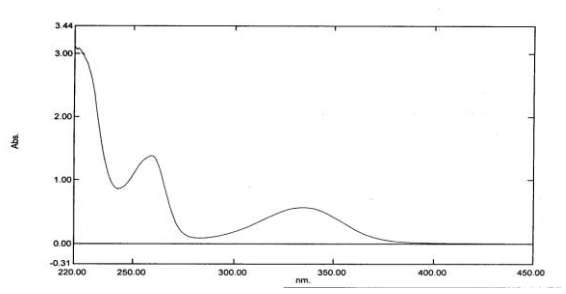
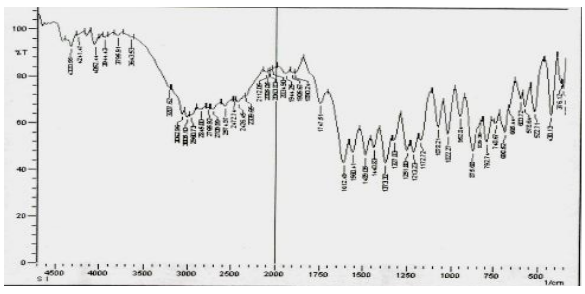
The purity of ketimines were ascertained by recording the melting point(uncorrected) and by analyzing them for carbon, hydrogen and nitrogen, The Physical and Analytical data of Schiff base, was studied from colour, melting point and elemental analysis.

III. Anti-Bacterial Study of the Ketimines:

The antibacterial studies are performed for all the Ketimines for strains *E. coli*, *S. typhe*, *S. aureus* and *B. subtilis* by disc diffusion method. **Experimental Procedure: [for details see our publication Int. J. Green Herbal Chem., commun., Dec. 2015].**

Results and Discussion:

All compounds gave satisfactory elemental analysis. Values are in the close agreement with the values calculated for expected molecular formulae assigned to these compounds and are in 5 % in statistics. The colour, physical constant and their purity was confirmed by TLC. Sample U.V. analysis of **S-I** is shown below.

Fig. 1. The UV-Vis spectra for a representative Schiff Base Compound (**S-I)**.	**Fig. 2** The FTIR Spectra for a representative Schiff Base Compound (**S-I)**.
	

The FTIR spectra for a representative Schiff Base, **S-I**, are reported in the **Fig. 2**.

In addition to this the compounds, **S-I** to **S-VII** were also characterized by UV-Vis, and FTIR spectral analysis were obtain and their structure were confirmed.

The condensation products of ketone and primary aromatic amines called as ketimines. In the present study, ketimines from 5-Chloro-2-hydroxy-4-methyl-acetophenone with Aniline, 4-Amino-phenol, 2-Amino-thiophenol, 4-Amino-thiophenol, 4-Chloro-2-nitro-aniline, 2-Amino-benzonitrile and 4-Amino-benzonitrile were synthesized and abbrivated respectively as **S-I** to **S-VII**. The progress of reactions was monitored by Silica gel-G TLC $60F_{254}$ Merck, visualized by UV cabinet or iodine vapour. These products were of yellow to brown in colour. The obtained products were purified by column chromatography on 60-120 Silica Gel. The purity of the compounds was ascertained by melting point determinations. Their TLCs were recorded on aluminium plates coated with silica gel. The experimental yields are in the range 90.21 % to 60.21 %.

All the studied Ketimine compounds showed 2-3 peaks in UV-Vis spectra in ethanol in the studied range 600 nm to 250 nm. In the UV-Vis spectral analysis of ketimines shows the two to three peaks in the studied range. These are attributed to n–π* and π–π* transitions due to presence of varied auxochrom group (auxochrome) and >C=N- group transitions and aromatic phenyl ring transition of moderate energy. The spectral data are in close agreement with the structures of the synthesized compounds.

The FTIR spectra of the studied Ketimines, **S-I** to **S-VII**, indicated the absorption at different frequencies as $\nu_{\text{-OH}}$ = 3100; $\nu_{\text{Ar-C-H}}$ = 1562 and 1460; $\nu_{\text{Ar-C-H}}$ = 2820 ; $\nu_{\text{>C=N-}}$ = 1641; $\nu_{\text{-NO2}}$ = 1446 and 1367 and $\nu_{\text{C-Cl}}$ = 810 and 772 for the respective functional groups as indicated.

These ketimines, **S-I** to **S-VII** were analysed by HPLC method and it is observed that the aniline and the ketimine products i.e. **S-I** to **S-VII** indicated different retention times.

In the present work, Ketimines are synthesized by conventional and microwave method and details are as below,

Table 1: Comparative data of Physical and Analytical data for Ketimines, S-I to S-VII, derived from 5-Chloro-2-hydroxy-4-methyl-acetophenone.

ID	Aniline used	Mol. Wt. of Aniline	Product, Mol. Wt.	Conventional Method		Microwave Synthesis	
				Product Wt., gm.	% Yield*	Product Wt., gm.	% Yield*
S-I	Aniline	93.13	259.63	5.72	66.75	6.29	72.80
S-II	4-Amino-phenol	109.13	275.63	5.75	63.86	6.14	66.91
S-III	2-Amino-thiophenol	125.19	291.69	5.50	57.55	2.92	60.35
S-IV	4-Amino-thiophenol	125.19	291.69	6.85	71.23	4.15	85.65
S-V	4-Chloro-2-nitro-aniline	172.57	339.07	7.35	65.12	4.02	71.21
S-VI	2-Amino-benzonitrile	118.14	284.64	5.18	55.38	5.70	60.21
S-VII	4-Amino-benzonitrile	118.14	284.64	6.85	72.34	8.54	90.21

***** isolated yield

The present yields obtained were compared with that of the conventional method(**Table-1**) and it is observed that these yields(by microwave method) are on higher side. Similar observations are reported by many researchers[36-38].

Anti-Bacterial Study of the Ketimines:

The antibacterial studies are performed for all the Ketimines for strains *E. coli*, *S. typhe*, *S. aureus* and *B. subtilis* by disc diffusion method.

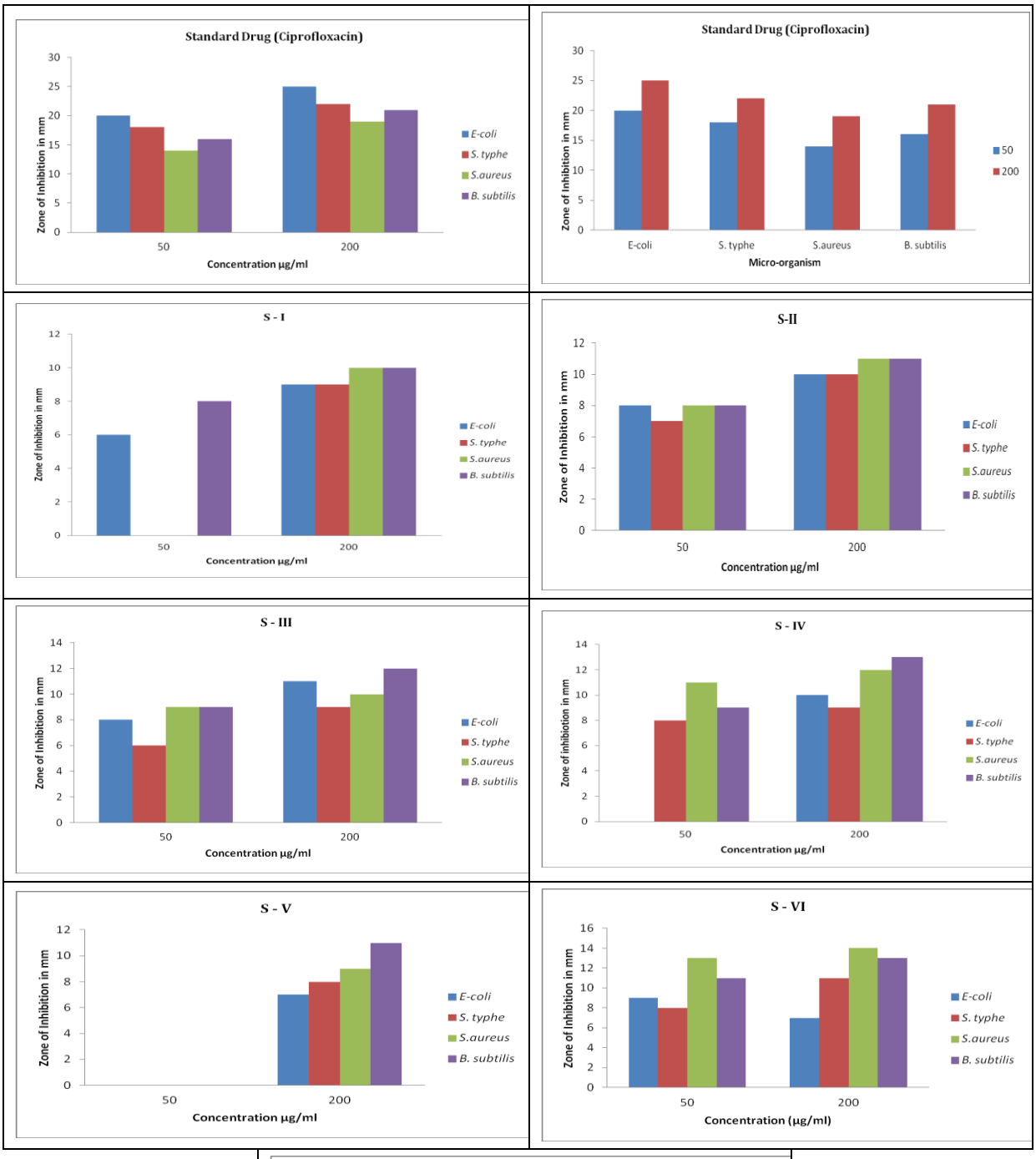
Experimental Procedure:

To study the antibacterial activity of Ketimines or Schiff Bases following setup was used. Newly synthesized compounds were screened for their antibacterial activities against four strain of bacteria *E. coli*, *S. typhe*, *S. aureus* and *B. subtilis* using disk diffusion method[39-40]. Inhibitory zone diameters for disks were measured in mm and compared with control disk. The results of anti-bacterial activity are depicted in **Table-2.**

Table-2. The Anti-bacterial activity screening for Synthesized Ketimines S-I to S-VII, derived from 5-Chloro-2-hydroxy-4-methyl-acetophenone.

Name of Strain→	***E. coli***		***S. typhe***		***S. aureus***		***B. subtilis***	
ID ↓	**Concn. (mg/ml)**		**Concn. (mg/ml)**		**Concn. (mg/ml)**		**Concn. (mg/ml)**	
	50	**200**	**50**	**200**	**50**	**200**	**50**	**200**
S-I	06	09	-	09	-	10	08	10
S-II	08	10	07	10	08	11	08	11
S-III	08	11	06	09	09	10	09	12
S-IV	-	10	08	09	11	12	09	13
S-V	-	07	-	08	-	09	-	11
S-VI	09	07	08	11	13	14	11	13
S-VII	08	09	10	12	11	13	11	11
Standard Drug (Ciprofloxacin)	20	25	18	22	14	19	16	21
+ ve control Distilled water)	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**
- ve Control (DMSO)	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**

The graphical representation of the anti-bacterial studies is as given below...



Achievements from the Project:

1. The literature on the synthesis of ketimines using Ketone with aniline and substituted anilines is updated.
2. Synthesize and characterize the ketimines from the 5-Chloro-2-hydroxy-4-methyl acetophenone an aromatic ketone with seven different aromatic amines viz. Aniline, 4-Amino-phenol, 2-Amino-thiophenol, 4-Amino-thiophenol, 4-Chloro-2-nitro-aniline, 2-Amino-benzonitrile and 4-Amino-benzonitrile.
3. The ketimines were purified and characterized by elemental analysis, colour, physical constant and TLC with varied spectroscopic methods such as UV-Vis, FTIR and HPLC.
4. The antibacterial activities of ketimines derivatives were determined.
5. All the above said Ketimines were tried using the microwave technique and their yields are compared.

Glimpses of the Anti-bacterial Study:

S-I compound is active at 200 mg/ml against all studied bacterial strains and inactive against lower conc. 50 mg/ml, active against ***S. typhe*** and ***S. aureus.***

S-II and **S-III** is active against all bacteria studied at all concentrations.

S-IV active against all bacteria studied except at low conc. against ***E. coli***.

S-V active against all bacteria studied at high conc. except at low conc. it is inactive against all bacteria studied.

S-VI and **S-VII** is active against all bacteria at all conc. and it is marked that their activities are somewhat closer to that of standard drug compared, Ciprofloxacin.

Contribution to the Society: (Give Details)

1. Society will refer this data, and the work done based on this topic and may be helpful in technical aspects either the one way or other.
2. A drug designer/ biochemist or a medicinal chemist may take the help of the results of antibacterial study in the present work or the suggested the strains which molecule or intermediate may be active.
3. Same study may be used by chemists to develop some newer compounds of future use which will serve the society in their development (This study may serve as a basis for their further planning).

Three research publication(proposed titles given below) are communicated to varied Scientific Journals and will be uploaded on our link (-http://khadsecollege.in/07_Reserach/default.aspx) and then see Menu-Research-Research Papers after publication.

Proposed Titles:

a) C. J. Patil, S. B. Salve and H. A. Mahajan, (Der. Chemica Sinica., commun., Dec. 2015) **Studies on Synthesis of Aromatic Schiff Bases. Part-V**. Conventional Synthesis and Characterization and Biological activity of Ketimines from 5-Chloro-4-methyl-2-hydroxy-acetophenone with substituted anilines.

b) C. J. Patil, S. B. Salve, C. A. Nehete and H. A. Mahajan, (Der. Pharma. Chemica., commun., Dec. 2015) **Studies on Microwave Synthesis of Aromatic Schiff Bases. Part-VI**. Microwave Synthesis and Characterization of Ketimines from 5-Chloro-4-methyl-2-hydroxy-acetophenone with substituted anilines.

c) C. J. Patil, Manisha C. Patil, S. B. Salve, H. A. Mahajan and Dhiraj Kolhe, (Int. J. Green Herbal Chem., commun., Dec. 2015) **Ketimines and Biological Screening: Part-3**. Antibacterial Screening of Novel Ketimines from 5-Chloro-4-methyl-2-hydroxy-acetophenone.

Conclusion:

Seven Ketimines were synthesized and characterized on the basis of analytical and spectral data. These compounds will be useful as building block by organic researchers in the near future. Screening of these compounds against pathogenic microorganism reveals that these compounds have the capacity of inhibiting metabolic growth of some microorganisms to different extent. The antimicrobial activity of the compound is also dependent on the nature of substituent present on the aromatic ring.

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