SYNTHESIS OF SCHIFF BASES FROM NATURAL PRODUCTS AND THEIR REMARKABLE ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY

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Abstract

Schiff bases have been synthesized by the condensation of primary amine with aldehyde under organic solvent free condition. The reaction is catalyzed by natural acid found in different natural products like tamarind and lemon. This reaction is very simple and has many benefits because of cheap catalyst, high yield of product, simple experimental conditions and easily available natural products. Many methods of Schiff base synthesis are reported but our objective is that to utilize such method and reagents which is environment friendly along with good yield. Most organic reactions utilized organic solvents and acids in which some are curse for environment because of this reason we did not use any organic solvent and acid. In this method Benzaldehyde is reacted with Aniline and Urea in the presence of natural acid extracted from tamarind and lemon to give Schiff bases SP-5 (Benzylidene aniline) and SP-18 (Benzylidene urea). The products (SP-5 and SP-18) were identified by various spectroscopic techniques such as IR, Mass and ^{1}H-NMR. These products also showed significant antibacterial, antifungal and antioxidant activities. The synthesis of SP-5 from tamarind and SP-18 from lemon is a new environmental friendly method for the synthesis of Schiff base. The present work revealed that Schiff bases with potential biological activity can easily be synthesized in the presence of natural acids as a catalyst. This method is effective, give good yield, time saving and easily approachable to everyone.

Introduction

The use of toxic and hazardous solvents in organic synthesis is considered a very important threat for the health and safety of chemist and environmental pollution. The majority of solvents are organic chemicals with hazardous and toxic properties. These solvents are expensive and their by-products are also toxic for environment. Water is universal solvent; we use it as "greener" solvent (non-toxic, benign to environment). It is very important to use water as a solvent because of easily availability and in-flammability. Schiff bases are biological active compounds they possess a lot of biological activities such as anticancer (Popp, 1961) , plant growth inhibitors (Imrie *et al*., 2007), insecticidal (Verma *et al.*, 2004), antidepressant (Gupta *et al*., 2006) ,antibacterial (Shah *et al*.,1992), anti-inflammatory (Hadjipavlou-litina and geronikaki,1996), anti-tuberculosis (Solak and Rollas, 2006), antimicrobial (Wadher *et al*.,2009) and anticonvulsant (Cates and Rasheed, 1984) activity. Due to such significant biological activities organic chemist are interested to synthesize Schiff bases. There are number of organic methods by which Schiff bases can be synthesized but these methods are harmful to environment as many organic reagents are toxic and carcinogenic. Greener methods for the synthesis of Schiff bases are also reported (Hossein *et al*., 2006; Bandale *et al*., 2011) but all these methodologies have some drawbacks like long reaction time, special conditions, special apparatus, cost of dehydrating agent etc. To reduce cost of catalyst herein we are reporting an environment friendly method for the synthesis of Schiff bases in which we use natural acids found in tamarind and lemon as a catalyst. This method is economical, short reaction time, free from organic solvent and give high yield of product.

Tamarind: *Tamarindus indica* (*tamarind*) is best described as sweet and sour in taste. It contain plant acid (16-18%) composed mainly of d-tartaric acid (up to ca.18%), with minor amount of l-malic acid. Other constituents include polyphenolics (catechin, epicatechinand procyanidin), flavonoids, sugar (20-40%), pectin protein (2.8%), fat, vitamin (B1 and C) minerals (Ca, K, P, etc) and tartrate (Popenoe, 1974).

We have used "extract of tamarind" as natural catalyst for synthesis of Schiff base. As tamarind extract is acidic in nature (pH = 3) and contain tartaric acid (8-12%) mainly along with citric and malic acid, so it works as an acid catalyst for Schiff bases formation.

Lemon Juice: *Citrus aurantium, Citrus indica, Citrus limonium* are some important species of citrus family commonly known as Lemon. Its juice is slightly turbid yellowish liquor, possessing a sharp, acid taste and grateful odour. We used here lemon juice as natural catalyst for synthesis of Schiff base .The main ingredients of lemon juice are mineral (0.3%), fat (0.9%),Vitamin-C (0.5%), protein (1%) Citric acid (5-7%), Carbohydrates (11.2%), moisture (85%) and some other organic acids. As lemon juice is acidic in nature (pH=2-3) and % of

Citric acid (5-7%) is more than other acids, it works as an acid catalyst for the synthesis of Schiff bases (Patil *et al*., 2011).

Materials and Methods

Benzaldehyde, Aniline and Urea were obtained from BDH/RDH/Sigma. Melting points were determined with a Gallenkamp melting point apparatus. IR (Infrared) spectra were recorded on a Jasco-302-A spectrophotometer. The EIMS (Electron impact mass spectrometry) were scanned on a Jeol-JMS HX-110 mass spectrometer. The ^{1}H-NMR (Nuclear magnetic resonance) spectra were recorded on a Bruker spectrometer operating at 500 MHz. The chemical shift values are reported in δ (ppm) relative to TMS (Tetra methyl silane) as internal standard. Purity of the products were checked by TLC (Thin layer chromatography).

General procedure for the extraction of Tamarind water: Fresh tamarind was obtained from market, socked it in water and stayed for overnight. We filtered the tamarind water and obtained clear filtrate and used it as a catalyst.

General procedure for the extraction of Lemon juice: Fresh lemon was cut by using knife and then pressed by hand using presser and extract juice then juice was filtered through filter paper to remove solid material then obtained a clear juice used as catalyst.

General procedure for the synthesis of Schiff base: The synthesis of Schiff base is described as a representative example: A mixture of benzaldehyde (1.2mL/11.77m.mol) and Aniline (1.1 mL/12.067m.mol) and 0.5 mL tamarind water was stirred at room temperature for 1 hour with monitoring by TLC. Then the reaction mixture was filtered and the pure crystalline product recovered by recrystallization with ethanol. The experimental procedure data of Schiff bases (SP-5 and SP-18) is given in Table 1 and their spectral data is given in Table 2.

Biological Assay: For Antibacterial and Antifungal activity the isolated bacterial and fungal strains were obtained from the department of Microbiology, Federal Urdu University of Arts, Science and Technology, Karachi-Pakistan.

Screening of Antibacterial activity: Antibacterial activity was performed by using agar-well method. Autoclaved Muller Hinton broth was used to keep the bacterial culture in log phase for 2 hrs with constant agitation and subsequently wells were dug onto Muller Hinton Agar. Later, 10 microliters of culture were poured into the wells (Perez *et al*., 2009). 10 mg/mL of the test compounds (SP-5 and SP-18) were taken for activity and all plates were incubated at 28$\pm$ 2°C for 24 -48 hours and after incubation diameter of zone of inhibition was noted (Sherwani *et al*., 2012). Gentamicin antibiotic was used as a control.

Screening of Antifungal activity: All the fungal isolates were checked for purity and maintained on Sabourd Dextrose agar (SDA) at 4^{0}C in the refrigerator until required for use. Antifungal activity was determined by using agar-well method. Autoclaved distilled water was used for the preparation of fungal spore suspension and transferred aseptically into each SDA plates (Wuthi-udomlert and Vallisuta 2011). 10 mg/mL of the test samples (SP-5 and SP-18) were taken for activity all plates were incubated at 28$\pm$ 2°C for 24 -48 hours and after incubation diameter of zone of inhibition was determined (Sherwani *et al*., 2013). Gresiofulvin antifungal agent was used as a standard.

Determination of Minimum inhibitory concentration (MIC): Minimum inhibitory Concentration (MIC) of SP 5 and SP 18 were determined by Micro broth dilution method using 96-well microtitre plate. Stock solution of 100 mg/mL of SP 5 and SP 18were prepared in distilled water. Two fold serial dilutions of test samples was made in 100 µl broth and subsequently 10 µl of two hour refreshed culture matched with 0.5 Mac Farland index was added to each well. One well served as antibiotic control while other served as culture control. Microtitre plate was incubated for 24 hours at 37 ºC. The MIC was read as the well showing no visible growth.

Determination of Antioxidant activity: For Antioxidant activity DPPH (1, 1-diphenyl-2-picrylhydrazyl) was prepared in ethanol (300 µM). The activity was determined by using the procedure described by (Lee *et al*., 1998). 10 µL of samples SP 5 and SP 18 and 90 µL solution of stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH) was added in 96 - well microtiter plates and incubated at 37º C for 30 minutes. Absorbance was measured at 515 nm by using a spectrophotometer. Percent inhibition of radicals by treatment of test sample was determined by comparison with a DMSO treated control group.

$$\% \text{ Inhibition} = \frac{(\text{absorbance of the control} - \text{absorbance of the test sample})}{\text{Absorbance of the control}} \text{ x } 100$$

Ascorbic acid was used as a standard. The EC_{50} value calculated denotes the concentration (in µg/ml) of sample required to scavenge 50% of DPPH.

Results and Discussion

It was observed that Schiff bases SP-5 (Benzylidene aniline) and SP-18 (Benzylidene urea) have been synthesized (Fig. I) by the condensation of benzaldehyde with aniline and then with urea. The carbonyl group in the benzaldehyde is electrophilic in nature whereas amino group in aniline and urea is nucleophilic. In the condensation reaction first step is the protonation of carbonyl oxygen. In this method natural acids found in tamarind extract and lemon juice were used as a catalyst to protonate carbonyl oxygen and under solvent free condition good yield of SP-5 and SP-18 were obtained. The product SP-5 was obtained as brown colour its IR (KBr) spectrum showed the characteristic peak of imine linkage at 1625 (C = N) cm^{-1}its mass spectrum showed the molecular ion peak at m/z 181.2 having molecular formula $C_{13}H_{11}N$. The ^{1}H-NMR ($CDCl_3$) spectrum also justify its structure by showing the signal of aromatic protons at δ 7.20-8.00 (m, 10H) and the characteristic peak of imine proton at δ 8.45 (s, 1H, HC=N). The product SP-18 was appeared as yellowish white in colour its spectral data also justify its structure by showing the following signals IR (KBr): 1640 (C = N) cm^{-1},^{1}H-NMR ($CDCl_3$): δ 7.1-7.5 (m, 5H, Ar-H), 8.24 (s, 1H, HC=N), EIMS (m/z) 148.1 with molecular formula, $C_8H_8N_2O$. Schiff base SP-5 (Majed M.H., 2009) and SP-18 (Kshash *et al*., 2011) has been synthesized earlier by organic method. The synthesis of Schiff base SP-5 from tamarind and SP- 18 from lemon is a new greener method for the formation of Schiff base. During the experiment it was found that no product was obtained in the absence of catalyst, therefore the role of tamarind extract and lemon juice in catalyzing the reaction is very important for the formation of Schiff base. This fruity method is not only cheaper but also time preventing just take 35 to 60 minutes to complete the reaction under solvent free condition with significant yield.

Benzaldehyde + Aniline → Benzylidene aniline (SP-5)

Benzaldehyde + Urea → Benzylidene urea (SP-18)

Fig. I. Reactions to show synthesis of Schiff bases catalyzed by Natural products.

Table1. Experimental procedure data of Schiff bases.

S.No	Reactant 01	Reactant 02	Catalytic natural food product	Product Codes	Colour of Product	Time (min)	m.p. ^{0}C	Yield %
01	Benzaldehyde (1.2ml/ 11.77m.mol)	Aniline (1.1ml/ 12.067m.mol)	Tamarind water (0.5ml)	stirring→ (SP-5)	Brown	60	102	85
02	Benzaldehyde (1.2ml/ 11.77m.mol)	Urea (0.6gm/ 12.067m.mol)	Lemon juice (0.5ml)	stirring→ (SP-18)	Yellowish white	35	74	89

Table2. Selected spectral data of Schiff bases.

Compound	^{1}H-NMR chemical shift HC=N(ppm)	EIMS m/z	IR stretch of C = N(cm^{-1})
SP-5	8.45	181.2	1625
SP-18	8.24	148.1	1640

Table 3. Antibacterial activity of SP-5 and SP-18 values are zone of inhibition (mm) and an average of triplicate and MIC (mg/ml).

Gram positive bacteria	Zone of inhibition in mm (mean±S.D)			MIC (mg/ml)	
	SP-5	SP-18	Standard Gentamicin	SP-5	SP-18
Bacillus cereus	-	15±1	>15	-	40
Bacillus subtilis	-	18±2	>15	-	84
Bacillus thruingiensis	-	-	>15	-	64
Staphylococcus epidermidis	-	-	>15	-	-
Streptococcus saprophyticus	-	-	>15	-	-
Corynebacterium diptheriae	-	-	>15	-	-
Corynebacterium hofmanii	-	-	>15	-	-
Corynebacterium xerosis	-	-	>15	-	-
Streptococcus fecalis	-	-	>15	-	-
Streptococcus pyogenes	-	-	>15	-	-
M. smegmatis	-	-	>15	-	-
Gram negative bacteria					
Enterobacter aerogenes	-	16±2	>15	-	24
Acinetobacter baumanii	-	10±1	>15	-	88
Vibrio cholerae	-	12±1	>15	-	84
Aeromonas hydrophila	-	12±1	>15	-	34
Campylobacter jejuni	-	-	>15	-	-
Campylobacter coli	-	-	>15	-	-
Escherichia coli ATCC 8739	-	-	>15	-	-
Escherichia coli	-	-	>15	-	-
E. coli multi drug resistance	-	-	>15	-	-
Klebsiella pneumoniae	-	-	>15	-	-
Salmonella typhi	-	-	>15	-	-
Salmonella paratyphi A	-	-	>15	-	-
Salmonella paratyphi B	-	-	>15	-	-
Shigella dysenteriae	-	-	>15	-	-
Serratia marcesens	-	-	>15	-	-
Campylobacter jejuni	-	-	>15	-	-
Campylobacter coli	-	-	>15	-	-
Helicobacter pylori	-	-	>15	-	-
Hemophilus influenzae	-	-	>15	-	-

Table 4. Antifungal activity of SP-5 and SP-18 values are zone of inhibition (mm) and an average of triplicate and MIC (mg/ml).

YEAST	Zone of inhibition in mm (mean±S.D)			MIC (mg/ml)	
	SP-5	SP-18	Standard Gresiofulvin	SP-5	SP-18
Candida albicans	13±1	-	>12	78	-
Candida albicans ATCC 0383	-	-	>12	-	-
Candida galbrata	-	-	>12	-	-
Candida tropicalis	-	-	>12	-	-
Candida kruzei	-	-	>12	-	-

YEAST	Zone of inhibition in mm (mean±S.D)			MIC (mg/ml)	
	SP-5	**SP-18**	**Standard Gresiofulvin**	**SP-5**	**SP-18**
Saccharomyces cerevisiae	-	-	>12	-	-
DERMATOPHYTES					
Microsporum canis	-	-	>12	-	-
Microsporum gypseum	14±1	-	>12	98	-
Trichophyton rubrum	-	-	>12	-	-
Trichophyton tonsurans	-	-	>12	-	-
Trichophyton mentagrophytes	-	-	>12	-	-
SAPROPHYTES					
Aspergillus flavus	11±1	-	>12	40	-
Aspergillus niger	17±0	-	>12	44	-
Fusarium specie	-	-	>12	-	-
Penicillium sp	-	-	>12	-	-
Rhizopus	-	-	>12	-	-
Helminthosporum	-	-	>12	-	-
Neurospora	-	-	>12	-	-

Table 5. Antioxidant activity of SP-5 and SP-18 EC_{50} value calculated denotes the concentration (in ug/ml) of sample required to scavenge 50% of DPPH.

S. No	Samples	% Inhibition ± SD	EC_{50} ug / ml
01	**SP-5**	69±0.01	937.5
02	**SP-18**	46± 0.01	-

The results of antibacterial activity (Table 3) indicate that SP-5 is inactive against gram positive and gram negative bacteria whereas SP-18 is active against gram positive bacteria like *Bacillus cereus, Bacillus subtilis*and gram negative bacteria like *Enterobacter aerogenes*, *Acinetobacter baumanii*, *Aeromonas hydrophila* and *Vibrio cholerae*. The results of antifungal activity (Table 4) showed that SP-5 is active against *Candida albicans* (yeast), *Microsporum gypseum* (dermatophyte), *Aspergillus flavus* and *Aspergillus niger* (saprophyte). The product SP-18 did not show any antifungal activity against yeast, dermatophyte and saprophyte. The results of antioxidant activity (Table 5) indicate that SP-5 (% inhibition 69) is a good antioxidant as compared to SP-18 (% inhibition 46).

Conclusion

The present study revealed that Schiff bases can simply be synthesized by natural acids found in natural products. This environmental friendly method is easily approachable. The products obtained by this method are high in quantity and showed remarkable antimicrobial and antioxidant activity.

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