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MICROWAVE ASSISTED SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL SCREENING OF DRUG BASED SCHIFF BASE SILVER COMPLEXES

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ABSTRACT

A series of drug based Schiff base ligands and their silver complexes were synthesized in alcoholic medium by microwave irradiation. The method yielded ligands and complexes in good quantities. The microwave synthesis has been found to be, relatively, simple and easier to carry out. The ligands and their silver complexes were characterized by elemental analysis, IR and NMR spectral data. The complexes were tested for antifungal and antibacterial activity. They have shown very good inhibition for fungal pathogens (C. albicans and Aspergillus niger) and pathogenic bacteria (S. aureus and E. coli).

KEYWORDS: Microwave irradiation, MIC, IR, Silver and Drug.

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INTRODUCTION

Almost all fungal organisms involved in human diseases are free-living, but humans have a high level of inborn immunity to infection due to innate natural barriers. In cases where the immune system has been compromised, either by disease or by therapies, fungal infection can become a problem. The emergence of new diseases and infections, such as Human Immunodeficiency Virus (HIV), Acquired Immunodeficiency Syndrome (AIDS), and the re-emergences of old ones, like Tuberculosis (T.B.), have led to an increase in the incidence of fungal infections. Also, the emergence of fungi which are resistant to the current prescription drugs is a matter of urgent concern, and it is this challenge that is driving the demand for new drugs. The use of metallic silver as an antimicrobial agent has long been recognized. The Greeks and Romans used silver vessels to keep water and other liquids from spoiling. Silver has a long traditional use in European folklore. It was believed to be an antidote to many maladies and monsters. In the middle ages, the use of silver tableware was thought to protect the wealthy from plague, with mortality amongst the rich being recorded as significantly lower than the poor. In 1884, Crede[1] prescribed a silver nitrate solution to protect against gonorrealophthalmia in neonates, a practice that is still in use today. In 1861, Thomas Graham discovered what he called “colloidal silver” and by the end of the 1900’s the use of silver was widespread. Polymeric Silver Sulfadiazine (SSD) is a combination of a sulfa drug and silver and it has both antibacterial and antifungal properties[2]. SSD is prescribed for the prevention and treatment of infection in patients with severe burns. Also, around this time, Johnson & Johnson introduced a silver impregnated cotton fabric wound dressing for the treatment of burns. Silver, either in its ionic, salt or complex form has been in the limelight of research which has been attributed mostly to its antimicrobial properties with minimal human toxicity. In view of these observations and in continuation of our interest in the synthesis of organic compounds for biological evaluation, we have described herein, facile synthesis of silver complexes using Schiff bases derived from drugs. They have been evaluated for their antifungal and antimicrobial activity. The objective is to develop new silver-drugbased antifungal agent to fight the fungal infections.

EXPERIMENTAL MATERIAL AND METHODS

All the chemicals and solvents used were of A. R. grade and were used without further purification. Thin layer chromatography was carried out on silica gel plates and the plates were scanned under 254 nm ultraviolet light. All the compounds were analyzed satisfactorily for C, H, N and S using micro analytical technique on ELEMENTAL analyzer at SAIF, COCHIN. The complexes were qualitatively tested for the presence of nitrate ion in the complex moiety. The synthesis of ligands and their complexes were carried out in open glass vessel in a Scientific Microwave Synthesizer Model: CATA-2R of capacity 32 liter with a power output of 850W and microwave frequency 2450 MHz. The temperature inside the reaction vessel was monitored by using a thermocouple. The microwave reactions were performed using on/off cycling to control the temperature. Completion of reaction was monitored by performing TLC and melting point. Infrared spectra of ligand and their silver complexes (in a KBr matrix) were recorded in the 4000- 350cm^{-1} region on Perkin Elmer FT-IR spectrophotometer and NMR on Brucker Avance II 400 FT NMR SPECTROMETER in DMSO using TMS as the internal standard. Antibacterial and antifungal activities of synthesized complexes were carried out in Bio-Genics, Research and Training Centre in Biotechnology, Hubli, and Karnataka.

Synthesis of Schiff base Ligands

The equimolar solutions of drug and salicylaldehyde (1:1ratio) in methanol were mixed thoroughly and small amount of glacial acetic acid was added. The mixture was subjected to microwave irradiation at an interval of 1 min at 450W for about 8-10 min. The progress of the reaction and purity of the products were monitored by TLC. After the completion of the reaction, the product was poured into ice cold distilled water and stirred well. The solid separated was filtered, recrystallized from ethanol and dried under reduced pressure (yield: 75-89%).

Sulpamethoxazole salicylaldehyde Schiff base ligand (L_1)

Melting point 204 °C, FT-IR (KBr, v cm^{-1}); 2976 (Phenolic OH), 1616 (CH=N), 1574 (Ring nitrogen), 1335 and 1158 (Symmetric and Asymmetric stretching vibrations of SO2NH). NMR (DMSO, ppm) 2.2ppm (CH_3 proton); 6.3ppm (Isoxazole ring proton); 6.9-8ppm (aromatic ring protons); 9ppm (Azomethine proton) 10.3ppm (Phenolic OH) and 10.7ppm (SO_2NH).

Trimethoprim-Salicylaldehyde Schiff Base (L_2)

Melting point 198 °C, FT-IR (KBr, v cm^{-1}); 3467 (NH_2); 3123 (Phenolic OH); 1590 (CH=N). NMR (DMSO, ppm) 3-4ppm (OCH_3 proton); 6.1 ppm (NH_2 protons); 6.6ppm (aromatic ring protons); 7.5 ppm (Azomethine proton)[3].

Synthesis of silver complexes

The synthesis of silver complexes was conducted in the absence of light and the products were also stored in the dark at all times.The equimolar solutions of ligand and silver nitrate in methanol were mixed thoroughly in 1:1 ratio for preparing C_1 and 2:1 for C_2. 0.1% KOH solution in methanol was then added to adjust the pH of the reaction mixture within 7-8. The mixture was irradiated in the microwave synthesizer at an interval of 1 min at 500W for about 15-20 min. The progress of the reaction and purity of the products were monitored by TLC. After the completion of the reaction, the solution was poured into cold distilled water and stirred well. The solid product obtained was filtered washed with water and finally with petroleum ether. The final product was dried under reduced pressure over anhydrous calcium chloride in a desiccator.

Ag – Sulpamethoxazole Salicylaldehyde Complex (C_1)

Melting point, 372 °C. FT-IR (KBr, v cm^{-1}); 1605 (CH=N), 1184 (Asymmetric stretching vibrations of

SO2NH), 579 (Ag-N stretch), 665 (Ag-O stretch). NMR (DMSO, ppm) 2.2ppm (CH_3 proton); 6.3ppm (Isoxazole ring proton); 6.9-8ppm (aromatic ring protons); 9ppm (Azomethine proton).

Ag–Trimethoprim-Salicylaldehyde Complex (C_2)

Melting point 278 °C. FT-IR (v cm^{-1}); 3466 (NH_2); 1552 (CH=N) 530 (Ag-N stretch), 778 (Ag-O stretch). NMR (DMSO, ppm) 3-4ppm (OCH_3 proton); 6.4 ppm (NH_2 protons); 6.6ppm (aromatic ring protons); 7.9 ppm (Azomethine proton)[4].

In - Vitro Antimicrobial Screening

The biological activities of synthesized complexes have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion methods respectively. The biological activities were done at 0.025, 0.050, 0.250, 0.500 and 1 mg/ ml in DMSO solvent by using bacteria-Staphylococcus aureus & Escherichia coli and fungi Aspergillus Niger & Candida albicans as follows:

Antibacterial and antifungal analysis[5, 6]

Composition of media used for antibacterial analysis is peptone-10g, sodium chloride 10g, yeast extract 5g, Agar 20g in 1000 ml of distilled water. Media used for antifungal analysis is sucrose 30g, sodium nitrate 2g, K_2HPO_4 1g, $MgSO_4.7H_2O$ 0.5g, KCl 0.5g, $FeSO_4$ 0.01g, Agar 20g. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37^0C for 18 hrs.The agar plates of above media were prepared and wells were made by using sterile cork borer of 6mm diameter in the plate. Each plate was inoculated with 18 hrs old cultures and spread evenly on the plate. After 20 min, the wells were filled with compound and antibiotic at different concentrations. All the plates were incubated at 37^0C for 24 hrs and the diameter of inhibition zone were noted. For antifungal activity the measurement the plates were incubated at 27^0C for 96 hrs.

RESULTS AND DISCUSSION

ELEMENTAL ANALYSIS DATA STUDIES

Micro analytical data of the complexes with proposed molecular formula are given in the following Table 1;

Table 1
Physicochemical data and elemental analysis

Ligand and Complex	Color	Molecular Formula	C%	H%	N%	S%
L_1	Pale yellow	$C_{17}H_{15}N_3O_4S$	(57.0) 57.08	(4.30) 4.22	(11.35) 11.12	(9.11 8.97
L_2	Yellowish Green	$C_{21}H_{22}N_4O_4$	(64.0) 63.15	(5.60) 4.80	(14.4) 14.10	-- --
C_1	Orange yellow	$C_{34}H_{28}O_8N_6Ag_2S_2$	(43.96) 41.34	(3.01) 2.71	(9.05) 9.15	(6.89) 6.68
C_2	Pale Brown	$C_{42}H_{42}O_{14}N_{10}Ag$	(49.50) 49.55	(4.12) 4.43	(13.72) 13.79	

L1- Sulphamethoxazole-Salicylaldehyde Schiff base; C1-Silver complex of L_1.
L2-Trimethoprim-Salicylaldehyde Schiff base; C_2-Silver complex of L_2

***The results obtained from elemental analytical measurements are in good agreement with calculated results from the empirical formula of each compound and confirms that the composition of the ligand and metal in the complexes corresponds to 1:1 for C_1 and 2:1 for C_2 with L_2:M_2 and L_2 :Mstoichiometry respectively*.**

Ir spectral studies

The IR spectra of the complexes were compared with those of the free ligands carefully in order to determine the mode of chelation. The FT-IR spectra of the investigated complexes contained almost all the absorption bands from the ligand and some new absorption bands which indicate the coordination of ligands with silver ion. The observed characteristic peaks (in cm^{-1}) in the IR spectra of Schiff base ligands and their silver complexes are mentioned in the synthetic part. The azomethine band in ligands L_1 and L_2 is shifted to lower wave number in the IR spectra of complexes which indicates the coordination of azomethine nitrogen to silver ion. The ligands show strong band at 2976.34 cm^{-1} in L_1 and 3123 cm^{-1} in L_2 due to phenolic -OH groups[7-9]. These bands disappear in the spectra of the complexes suggesting that the ligands are coordinated through the phenolic oxygen atom via de-protonation. The bands at 1335 cm^{-1} and 1158 cm^{-1} in the IR spectra of the ligand L_1 are assigned due to symmetric and asymmetric stretching vibrations of SO_2NH. The band appeared at 1335cm^{-1} in spectra of L_1 disappeared and the band at 1158 cm^{-1} is sifted to lower frequency in the spectra of its silver complex indicating the involvement of Nitrogen of SO_2NH in the complex formation[10]. The bonding of silver metal ion is further supported by the appearance of new absorption bands 579 & 530 cm^{-1} and 665 & 778 in the spectra of C_1 and C_2 which are assigned to Ag-N and Ag-O respectively.

^{1}hnmr spectral studies

The –OH proton of salicylaldehyde moiety resonated as a singlet at 10.3 and 10.7 in case of the ligands disappeared in the complex indicating the involvement of phenolic oxygen in coordination via de-protonation. The involvement of nitrogen of SO_2NH is further supported by the disappearance of the singlet at 10.7ppm due to SO_2NH proton in the ^{1}HNMR spectra of ligand L_1[11, 12].

Antimicrobial activity

The results of antibacterial and antifungal activities are presented as diameter of inhibition zones and as minimum inhibition concentration (MIC) in the tables 2, 3 and 4 respectively. The graphical representation of the comparative statement of inhibition zone of synthesized silver complexes with the standard parent drugs is also shown in the Fig. 2. Idemudia et al.[13], reported that

5mg/ml of Trimethoprim drug exhibited activity with 20mm diameter of inhibition zone against S. aureus while only 1mg/ml of its synthesized silver complex showed 11mm diameter of inhibition zone against same bacteria. Moreover 5mg/ml of Trimethoprim is found inactive against E.coli but only 1mg/ml of its synthesized silver complex exhibited activity with 13 mm diameter of inhibition zone against same bacteria. Bharti Jain et al[14] reported that 1mg/ml of Sulphamethoxazole drug and its Schiff base with salicylaldehyde exhibited biological activity with 10.2mm and 11mm diameter of inhibition zone against bacteria S. Aureus and E.coli respectively.

But 1 mg/ml of C_1 of same Schiff Base showed 11 mm and 17 mm diameter of inhibition zone against same bacteria. These observations suggest that synthesized silver complexes are better antibacterial agents as compared to parent drug. However, C_1 complex shows potent antifungal activities against C. albicans as well as A. niger with appreciable zone of inhibition diameter. But C_2 is inactive towards fungus C. albicans. It is also observed that in case of C_1 at 1mg/ml concentration, the diameter of inhibition zone is too big to measure which indicates that C_1 silver complex is significantly antifungal agent and inhibit the growth of fungi to a greater extent.

Table 2
The results of antibacterial activity are presented in the following table as diameter of inhibition zone in mm.

Pathogens Sample	Antibacterial activity									
	S. aureus					E. coli				
	0.025mg	0.050mg	0.250mg	0.500 mg	1mg	0.025mg	0.050mg	0.250mg	0.500 mg	1mg
C_1	0	0	5	8	11	0	0	10	14	17
C_2	0	0	8	10	11	0	0	8	10	13

Table 3
The results of antifungal activity are presented in the following table as diameter of inhibition zone in mm

Pathogens Sample	Antifungal activity									
	A. niger					C. albicans				
	0.025mg	0.050mg	0.250mg	0.500 mg	1mg	0.025mg	0.050mg	0.250mg	0.500 mg	1mg
C_1	0	13	19	24	*	15	18	28	33	*
C_2	0	0	10	16	20	0	0	0	0	0

***Inhibition zones were too big to measure.**

Table 4
The results of antibacterial and antifungal activity are presented inthe following table as minimum inhibition concentration (MIC) in mg/ml

Pathogens Sample	Antibacterial activities		Antifungal activities	
	S. aureus	E. Coli	A. niger	C. albicans
C_1	0.25	0.25	0.050	0.025
C_2	0.25	0.25	0.250	--

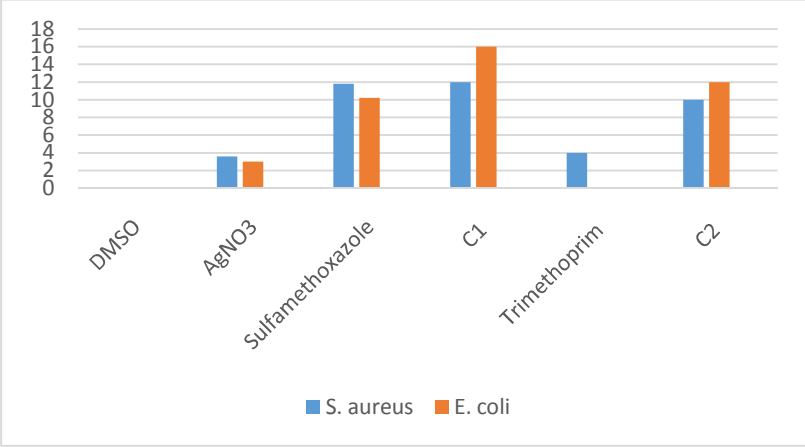

Figure 1
Comparative statement of Inhibition zone of compounds in mm

The above mentioned results reveal that both the synthesized complexes C_1 and C_2 show more antibacterial activity than their corresponding parent drug. However C_1-complex shows potent antifungal activities against C. Albicans as well as A. niger with appreciable zone of inhibition diameter. But C_2 is inactive towards fungus C. albicans. The Photographs of inhibition zone inhibited by Ligands and complexes on selected microorganisms are shown in the figure 2.

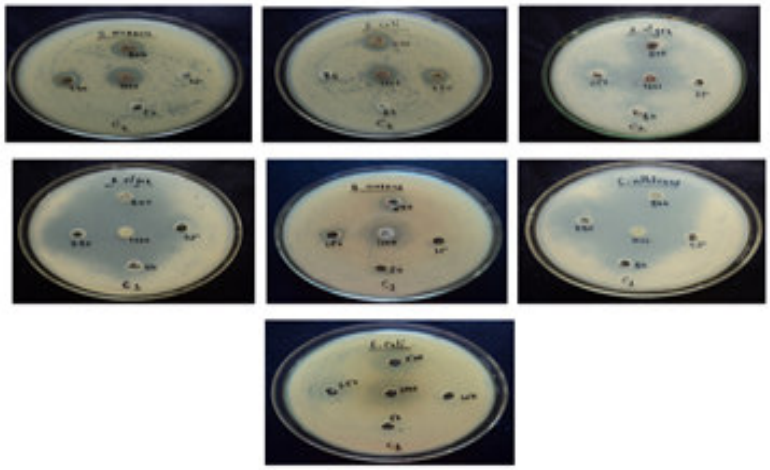

Figure 2
Photographs of inhibition zone inhibited by Ligands and complexes on selected microorganisms
On the basis of elemental, FTIR, and ^{1}HNMR the following structures have been proposed for the synthesized silver complexes C_1 and C_2 (Fig. 3).

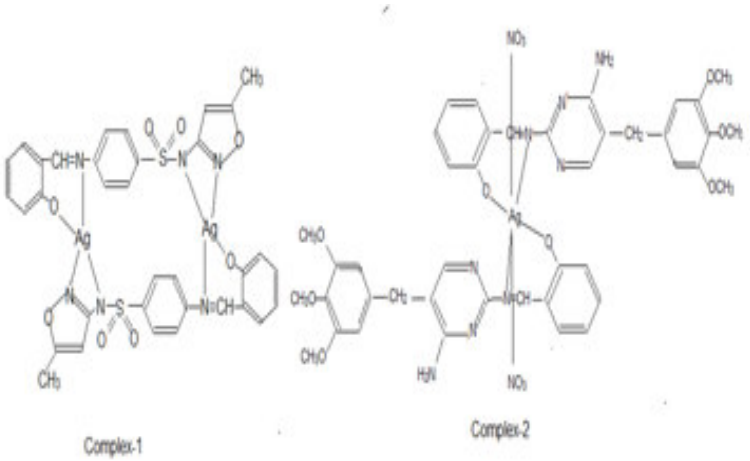

Figure 3
Proposed Structures of C1 and C2 Complexes

Further on the basis of the results of biological activities obtained above shows that both the synthesized Silver complexes C_1 and C_2 are having remarkable antibacterial activity than their corresponding parent drug. However C1-complex shows potent antifungal activities against C. Albicans as well as A. niger with appreciable zone of inhibition diameter. But C_2 is inactive towards fungus C. albicans. It is also observed that in case of C_1 at 1mg/ml concentration, the diameter of inhibition zone is too big to measure which indicates that C_1 silver complex is significantly antifungal agent and inhibit the growth of fungi to a greater extent. Antimicrobial activity of the green synthesized silver complexes proves the potential use of silver complexes in the area of medicine.

CONCLUSIONS

Microwave assisted synthesis has reduced reaction time from hours together to few minutes with better yield compared to classical synthesis methods. The synthesized Schiff base ligands are coordinated in tetra-dentate and tridentate manner with silver ion.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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