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A SIMPLIFIED GREEN CHEMISTRY APPROACH TO THE KNOVENEGAL CONDENSATION REACTION USING"GRIND STONE" CHEMISTRY AND STUDY OF THEIR ANTI BACTERIAL AND ANTIFUNGAL ACTIVITY

D.Bharathi*, N.Vimal, S.Soundhar, D.Dilli babu, K.Premavathi

Department of Pharmaceutical Chemistry, Jaya College of Paramedical Science, Chennai, Tamil Nadu, India.

ABSTRACT

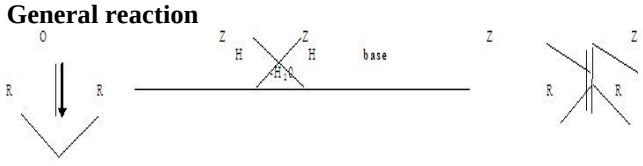
The proposed work was planned to synthesis ester using Knoevenagal condensation method by simplified green chemical approach. The mixture of aldehyde (1 m mole) & active methylene compound (1 m mole) & sodium carbonate (0.1 m mole) were ground together in a mortar at 26° c. The reaction mixture was solidified. The melting point, IR, HNMR & LC Mass spectral data of novel arylmethylidene compounds was prepared. The antibacterial and antifungal activity was also performed.

Key Words: Methylene compound, Sodium bicarbonate, Knoevenagal condensate, Antibacterial and Antifungal.

INTRODUCTION

In 1896, Knoevenagal reported benzaldehyde with ethyl aceoacetate condensed at room temperature in the presence of piperdine to give a bis compound, but that when the reaction was run in a freezing mixture, the product was acetoacetate. For this purposes, the Knoevenagal condensation is defined as the reaction between an aldehyde or ketone or any compound having an active methylene group. Brought about by an organic base or ammonia and their salts. The Knoevenagal condensation reaction is an organic reaction named after Emil Knoevenagal. It is a modification of the aldol condensation. A Knoevenagal condensation is a nucleophilic addition of an active hydrogen compound to a carbonyl group followed by a dehydration in which a molecule of water is eliminated. The product is often an alpha, beta conjugated enone [1-5].

General reaction



In this reaction the carbonyl group is an aldehyde or a ketone. The catalyst is usually a weakly basic amine. The active hydrogen component has the form of Z-CH2-Z or Z-CHR-Z for instance diethyl malonate, Meldrum's acid, ethyl acetoacetate or malonic acid. Z-CHR1R2 instance nitro methane, Where Z is an electron withdrawing functional group [6, 7].

MATERIALS AND METHOD OF PREPARATION

Table 1. Chemicals

S.NO	ALDEHYDE REACTANT	NAME OF THE MANUFCTURER
1	Acetaldehyde	Chem. Method
2	Benzaldehyde	Chem. Method
3	Formaldehyde	Chem. Method
S.NO	ACTIVE METHYLENE GROUP	NAME OF THE MNUFACTURER
1	Ethyl Aceto Acetate	Chem. Method
S.NO	SOLVENT	NAME OF THE MANUFACTURER
1	Water	Chem. Method
2	Carbon Tetrachloride	Chem. Method
3	Ethanol	Hayman Limited

Corresponding Author:- **D.Bharathi** Email:- bharathid@gmail.com

Table 2. Catalyst

S.NO	CATALYST	NAME OF THE MANUFACTURER
1	Zinc Carbonate	Chem. Method
2	Calcium Carbonate	Chem. Method
3	Sodium Carbonate	Chem. Method
4	Sodium Bicarbonate	Chem. Method
5	Pyridine	Chem. Method
6	Ammonium Chloride	Chem. Method

Table 3. Spectral analysis of products of Knoevenagal condensation

Compounds	IR				HNMR	LC-MASS
	C-H (s)	C=O(s)	C=C	C-H(b)		
A	2976	1731	1668	-	1.3 – 7.07	156.18
B	3073	1696	1635	-	1.3 – 8.00	218.25
F	-	-	1600	3400	1.3 – 7.00	142.15

Experimental section

Aldehyde, methylene compounds and sodium carbonate are purchased from SD fine-chem. limited and is of LR grade and used without further purification. Solvents were distilled before use. Reactions were monitored on TLS by comparison with the authentic samples. Melting points were determined by a buchi melting point apparatus. IR, HNMR and LC-MS were recorded on a Nicolet 400D FT-400 MHz Brucker spectrophometers and an Agilent technologies 1200 series instrument respectively [8-10].

General procedure

A mixture of an Aldehyde (2 mmole), active methylene compound (2 mmole) and sodium carbonate (0.2 mmole) were ground together in a mortar at 26 °c. The reaction mixture was solidified within 1-5 min after completion of the reaction. Water was added, stirred for a minute, filtered and dried. Recrystallisation was not necessary. The melting point, IR, HNMR and LC-mass spectral data of novel arylmethylidene compounds were prepared .Different products were obtained when different aldehyde were used. The details of the result with respect to Aldehyde used is as follows [11,12],

1. Acetaldehyde gives 2-acetyl-but-2 enoic acid ethyl ester (A)
2. Benzaldehyde gives 2-benzylidene 3 oxo butyric acid ethyl ester (B)
3. Formaldehyde gives 2-methylene 3 oxo butyric acid ethyl ester (F)

Data on anti bacterial activity

Procedure

The antibacterial activity of (A, B, F) against Klebsiella pneumonia, Escherichia coli and staphylococcus aureus antimicrobial activity antibacterial activity all the compounds were screened for their antibacterial activities against gram-positive bacteria such a staphylococcus aureus and also against gram-negative bacteria such as Klebsiella pneumonia, Escherichia coli bacteria strains at concentrations of 100, 200,300 and 500 µg/ml. Streptomycin was used as a reference standard. Petri plates and necessary glassware were sterilized in hot air oven at 190° c for 45 min. The Muelier Hinton agar and saline (0.82% NaCl) media were sterilized in autoclave (121° c, 15 psi, and 20 min). Inoculum was prepared in sterile saline (0.82%NaCl). The Muelier Hinton agar plates were prepared by the pour plate bacterial cells were cultured in Muelier Hinton agar plates and the compounds to be tested were dissolved in N,N-dimethyl-formamide (DMF) and were soaked in agar disc and the Petri plates incubated at 37° c for 24 h. The diameter (mm) of the zone of inhibition around each agar disc was measured and results were recorded. Compounds tested were found to have excellent anti-bacterial activity against Klebsiella pneumonia and Escherichia coli. However, they were found to have moderate activity against and staphylococcus aureus [13-15].

Scheme…

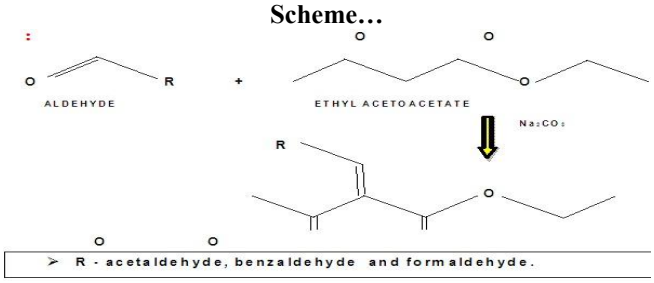


Table 4. Zone of Inhibition of Streptomycin

S.NO	COMPOUNDS	TYPE OF BACTERIA	Inhibition zone in mm for concentration of			
			100 µg/dl	200 µg/dl	300 µg/dl	500 µg/dl
1	STANDARD (Streptomycin)	Klebsiella pneumonia	14	18	23	32
		Escherichia coli	13	15	20	31
		Staphylococcus aureus	10	13	16	24
2	A	Klebsiella pneumonia	15	18	24	34
		Escherichia coli	12	11	20	30
		Staphylococcus aureus	9	12	15	22
3	B	Klebsiella pneumonia	13	14	22	30
		Escherichia coli	15	17	25	33
		Staphylococcus aureus	8	11	14	24
4	F	Klebsiella pneumonia	12	15	14	29
		Escherichia coli	12	19	17	25
		Staphylococcus aureus	13	20	22	29

Table 5. Zone of Inhibition of Mycostatin

S.NO	COMPOUNDS	TYPE OF FUNGI	Inhibition zone in mm for concentration of			
			100 µg/dl	200 µg/dl	300 µg/dl	500 µg/dl
1	STANDARD (Mycostatin)	Rhizoctonia solani	16	20	28	35
		Fusarium oxysporum	16	19	27	35
		Aspergillus niger	11	14	21	30
2	A	Rhizoctonia solani	17	22	30	38
		Fusarium oxysporum	15	16	22	30
		Aspergillus niger	11	12	20	29
3	B	Rhizoctonia solani	12	19	25	33
		Fusarium oxysporum	19	20	28	38
		Aspergillus niger	11	12	20	29
4	F	Rhizoctonia solani	15	15	26	34
		Fusarium oxysporum	12	20	25	34
		Aspergillus niger	17	22	30	38

Fig 1. Antibacterial activity of (A-B-F) against Klebsiella pneumonia, Escherichia coli and staphylococcus aureus

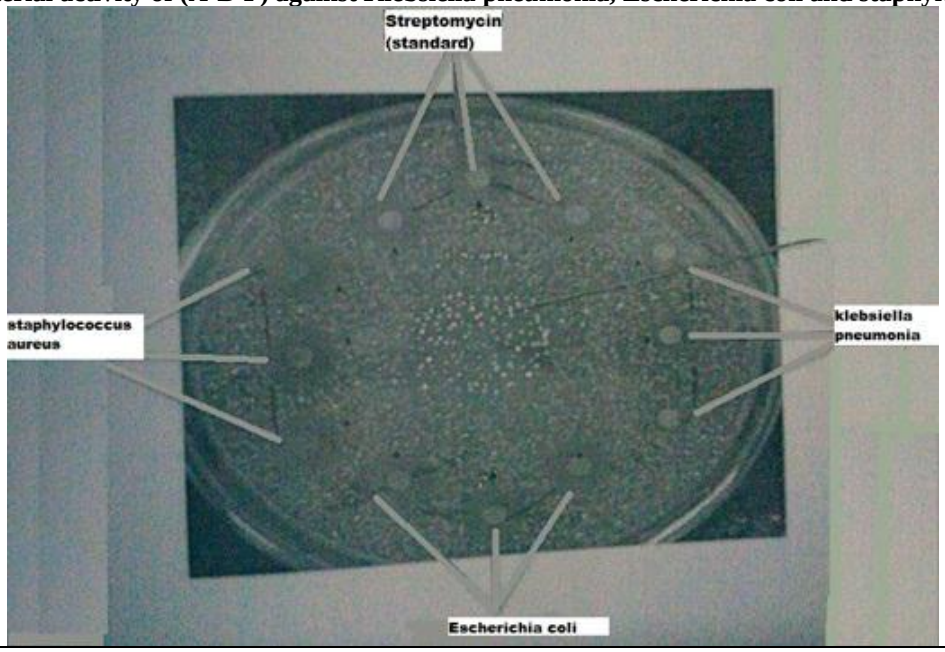
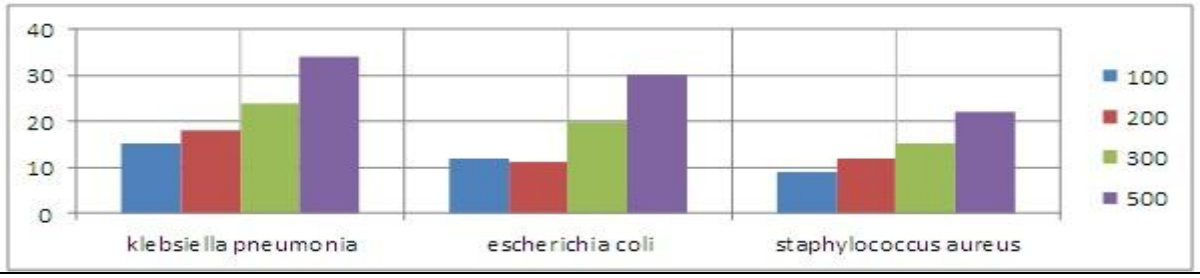
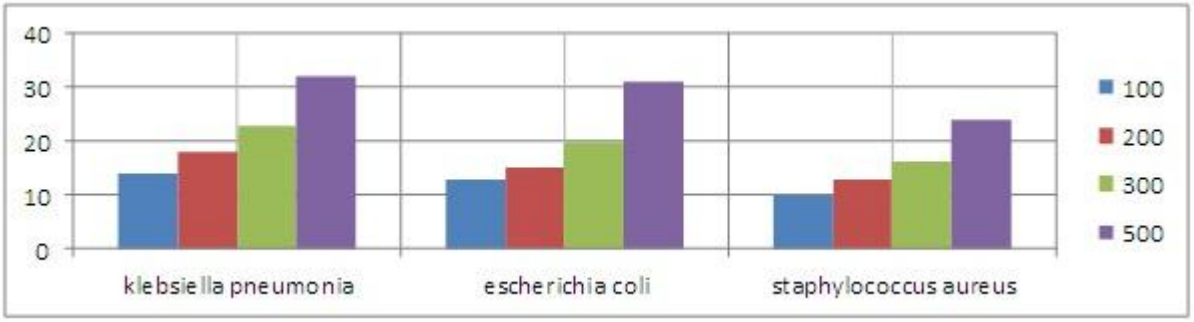


Fig 2.
Y AXIS : Concentration in µg/ml
X AXIS : Types of bacteria

(S)



(A)



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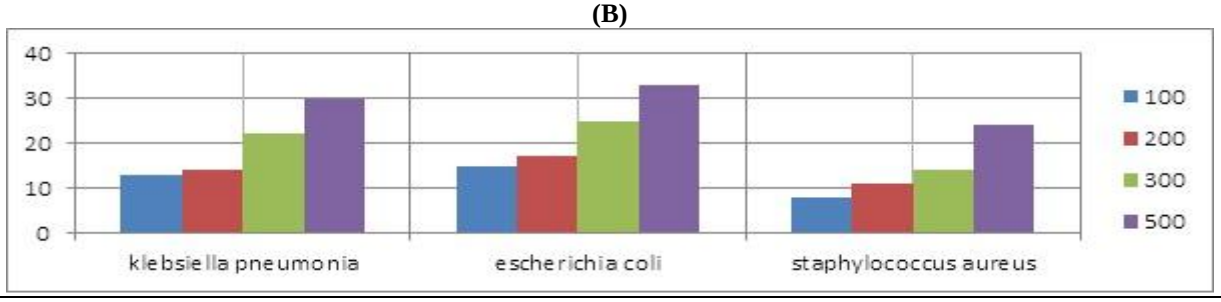

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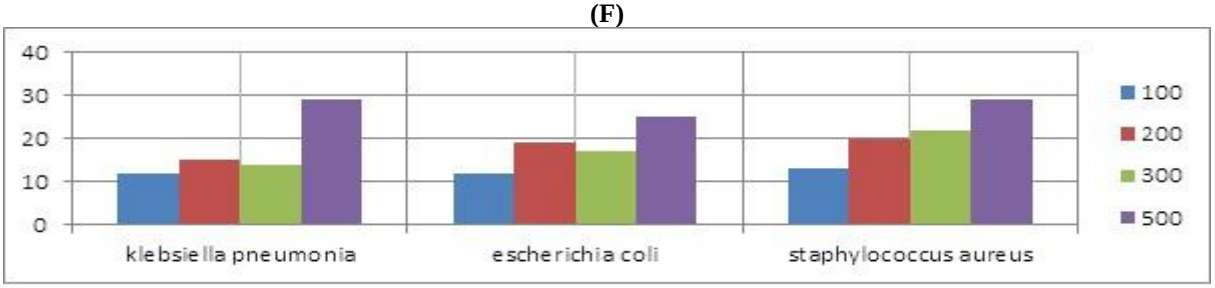


Fig 3. Antifungal inhibition of synthesized compounds – (A,B,F) and standard against various fungi

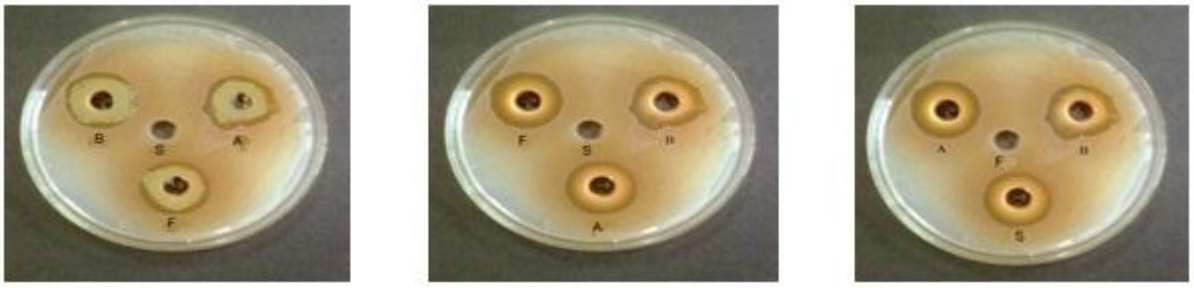
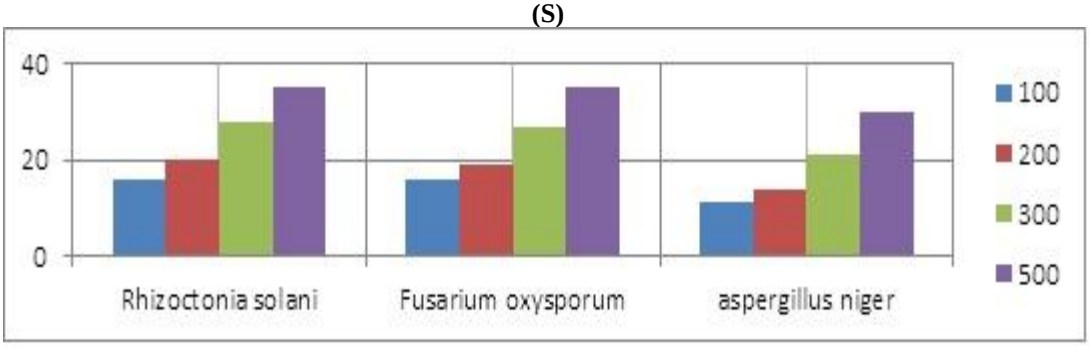
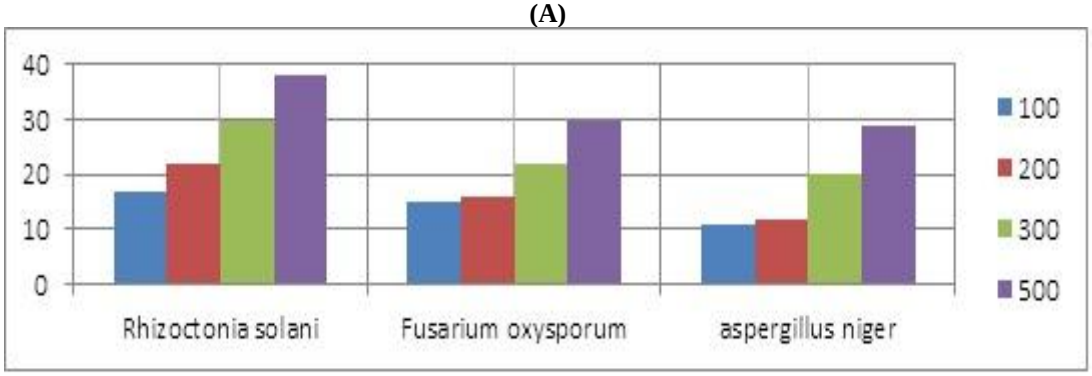


Fig 4.
Y AXIS : Concentration in µg/ml
X AXIS : Types of bacteria

(S)



(A)



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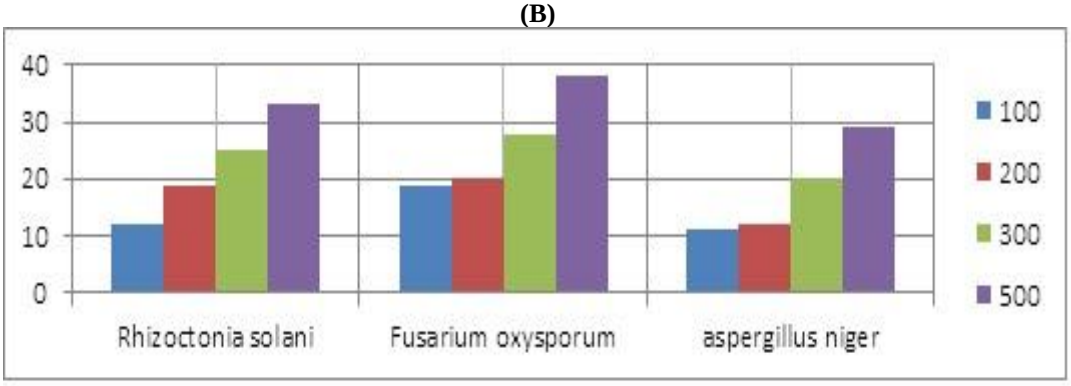
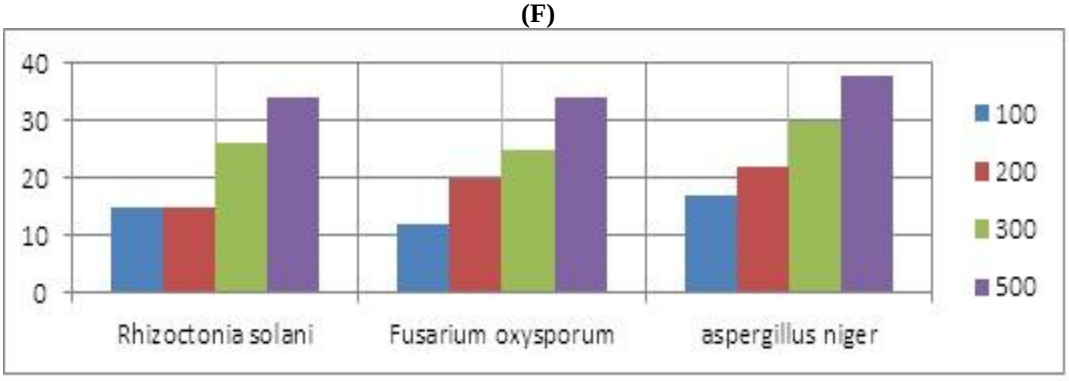


(F)



Data on anti fungal activity

Procedure

Antifungal activity of all the compounds synthesized were screened for antifungal activity against Rhizoctonia Solani, Fusarium Oxysporum and Aspergillus Niger at concentrations of 100, 200,300and 500 µg/ml. Mycostatin was used as a reference standard. Potato dextrose agar (PDA) was used as basal medium for test fungi. Glass Petri dishes used were sterilized. Sterilized melted PDA medium(45 °c) was poured at the rate of 15 ml into each Petri dish (90mm). After solidification of the medium, small portions of the mycelium of each fungus were spread carefully over the centre of each PDA plate with the help of sterilized needles. Thus , each fungus was transferred to a number of PDA plates, which were then incubated at (25±2)°c and ready for use after five days of incubation. Prepared discs of samples were placed gently on solidified agar plates, freshly seeded with the test organisms with sterile forceps. A control disc was also placed on the test plates to compare the effect of the test samples and to nullify the effect of solvent respectively. The plates were then kept in a refrigerator at 4°c for 24 h so that the materials had sufficient time to diffuse over a considerable area of the plates. After this, the plates were incubated at 37°c for 72 h. N,N-dimethyl formamide (DMF) was used as solvent to prepare desired solutions of the compounds and also to maintain proper control. The diameter (mm) of the zone of inhibition around each agar disc was measured and results were recorded. Compounds tested were found to have very good antifungal activity against Rhizoctonia Solani and Fusarium Oxysporum. However, they were found to have good activity against and Aspergillus Niger.

CONCLUSION

Knoevenagal condensation is a simple, efficient and less expensive method involving a reaction set up not requiring specialized equipment. It is a also a rapid, convenient and safe method working at room temperature by which excellent product yields are obtained in a short reaction time with or without solvent. The maximum yield product can be obtained only in acetaldehyde. On evaluating the pharmacological activities of yields, all the products exhibit comparable antibacterial and antifungal activity against the organisms tested.

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CONFLICT OF INTEREST

No interest

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