IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Antibacterial and Antifungal Activities of new unsymmetrical thiobarbituric acids and their Knoevenagel Products

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Abstract

The synthesized thiobarbituric acids and Knoevenagel products were evaluated for their antimicrobial activities against various pathogens *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* using cup plate agar diffusion method.

Introduction

Microbes are unique creatures that adapt to varying lifestyles and environment resistance in extreme or adverse conditions. Continuous change in genetic architecture of microbes it becomes a challenge for the society to find new chemical entities which can treat microbial infections.[1] The alarming rates of the growing emergence of antimicrobial resistance are major concern to the public health and scientific communities worldwide, especially in the field of multi drug resistant bacteria and fungi.[2,3] These trends have emphasized the urgent needs for new, more effective, less toxic and safe antimicrobial agents and the development of structurally new classes of antimicrobials with novel mechanisms of action as well as structural modifications to improve both their binding affinity and their spectrum of activity.[4]

Structural modification of antimicrobial drugs to which resistance are developed and has been proven to be an effective means of extending the lifespan of antifungal agents such as the azoles,[5] antiviral agents such as the non-nucleoside reverse transcriptase inhibitors[6] and various antibacterial agents including β-lactams and quinolones.[7] It is not surprising in response to antimicrobial resistance, major pharmaceutical companies have tended to concentrate their efforts on improving antimicrobial agents in established classes.[8,9,10]

Heterocyclic compounds are abundant in nature and are of great significance to life because their structural subunits exist in many natural products such as vitamins, hormones, and antibiotics,[11,12] hence, they have attracted considerable attention in the design of biologically active molecules[13,14] and advanced organic chemistry.[15,16] Also in the family of heterocyclic compounds nitrogen containing heterocycles such as azine,[17] diazine[18] are an important class of compounds in the medicinal chemistry and also contributed to the society from biological and industrial point which helps to understand life processes.[19]

In view of proven potentially of thiobarbituric acid analogues which contain 4,6-dioxo-2-thioxopyrimidine pharmacophore, it has been planned to synthesize various analogues of 4,6-dioxo-2-thioxopyrimidines containing other interesting aldehydes responsible for antimicrobial activity.[20] The medicinal importance of pyrimidine derivative such as barbituric and thiobarbituric acids are play vital role among various heterocyclic compounds due to their antineoplastic, anti-viral[21] and antibiotic and antiinflammatory activities. The diverse of biological activity and coverage of abroad chemical space make barbituric and thiobarbituric acid derivatives excellent target compounds for organic and medicinal chemists.[22-28]

Among the most important C-5 functionalized barbituric acids and thiobarbituric acids are the 5-benzylidene or 5-methylene derivatives, usually produced by their reaction with benzaldehydes or triethyl orthoformate, respectively. Out of these, a large variety of 5-arylidene barbituric acids and thiobarbituric acids have been biologically evaluated and several compounds of this class were found to possess antimicrobial activity against a number of Gram-positive and Gram-negative bacteria, as well as against fungal strains.[29] Methylene barbituric acids and thiobarbituric acids are most often used as intermediates for the synthesis of other derivatives.[30,31] Within these, hydrazone barbituric acids have the ability to inhibit fungal growth in the mm range.[32] In addition, through the introduction of urea, thiourea, guanidine, hydrazine or hydroxyl amino groups to barbituric acids and thiobarbituric acids, new bioactive compounds can be obtained.[33,34]

The present chapter deals with the evaluation of antimicrobial potential of newly synthesized thiobarbituric acids[35] and their Knoevenagel products[36] by cup plate agar diffusion method.[37]

Results and discussion

The compound or substance to be evaluated must be brought in an intimate contact with the test organisms against which activity is to be estimated. Favorable conditions like nutritional media, temperature, incubation time etc. must be provided to offer a maximum opportunity for optimum growth of the organisms in absence of antimicrobial agents. There should be method for measuring antimicrobial response obtained by antimicrobial agents. Various methods have been proposed and adopted for the measurement of antimicrobial activity.[38]

In present study, the newly synthesized thiobarbituric acids and their Knoevenagel products were screened for their antimicrobial activities against some selected microorganisms includes *B. cereus*, *E. coli*, *S. aureus* and *C. albicans* using cup plate agar diffusion method.[61] The antibacterial activity and inhibition effect of the compounds on the growth of various bacterial organisms is summarized in Table **1** and **3**.

The synthesis of thiobarbituric acids (**3a-o**) were screened for their antibacterial activities (Table **1,** Plate **1-14**). Almost all compounds (**3a-o**) were found sensitive and **3d** (Plate **1**), **3h** (Plate **2**) and **3o** (Plate **4**) are highly sensitive against the organisms *B. cereus*. Most of the synthesized title compounds were found highly resistant against *E. coli* except **3g, 3h, 3k, 3l, 3n & 3o** (Plate **8-9**) were sensitive. Almost all compounds (**3a-o**) were found highly resistant against fungal strain *C. albicans* except compound **3e** (Plate **12**) alone sensitive. The name of the compounds along with their numbers and codes are given in Table **2**.

The newly synthesized Knoevenagel products (**3a-x**) were found highly good antibacterial activities (Table **3,** Plate **15-22**). Inhibition zone record of the compounds shown that, almost all compounds (**3a-x**) were highly sensitive while compounds **3g** (Plate **17**), **3i** (Plate **18**), **3m, 3t** & **3x** (Plate **16**) were found highly sensitive against the organisms *S. aureus*. We also observed that all compounds were found highly resistant against *E. coli*. Compound **3b** (Plate **19**) was sensitive against *E. coli* respectively. The name of compounds along with their numbers and codes are given in Table **4**.

Table 1. Antibacterial and antifungal activities of thiobarbituric acids against microorganisms.

Compound No.	Code	Microorganisms					
		B. cereus	Plate No.	*E. coli*	Plate No.	*C. albicans*	Plate No.
3a	POTTB	15	1	--	7	--	11
3b	PMTTB	12	1	--	7	--	11
3c	POCTB	13	1	--	7	--	11
3d	PMCTB	17	1	--	8	--	12
3e	PTOTT	13	2	--	8	12	12
3f	PTMTT	13	2	--	8	--	12
3g	PTOCT	13	2	13	8	--	12
3h	PTMCT	17	2	13	-	--	13
3i	PAOTT	12	3	--	-	--	13
3k	PAMTT	13	-	13	-	--	13
3l	PAOAT	12	3	12	-	--	14
3m	PAOCT	13	3	--	-	--	14
3n	PAMCT	13	-	12	-	--	14
3o	PCETB	16	4	13	9	12	14
Control		11	5	--	6	--	10

(Concentration 100 µg/ml) (Inhibition zone in mm)

(--): Resistant (10 mm and less); sensitive (11-13 mm); Moderately sensitive (14-15 mm); Highly sensitive (16 mm and above)

Plates: Antimicrobial activity of thiobarbituric acids against various microorganisms

***Bacillus cereus*:**

Plate-1 (3a, 3b, 3c, 3d)

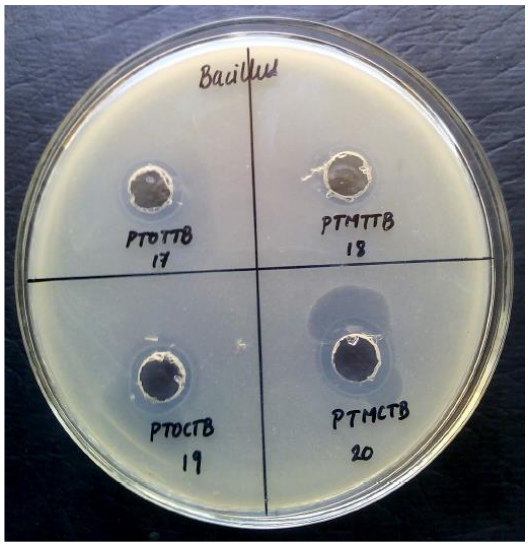

Plate-2 (3e, 3f, 3g, 3h)

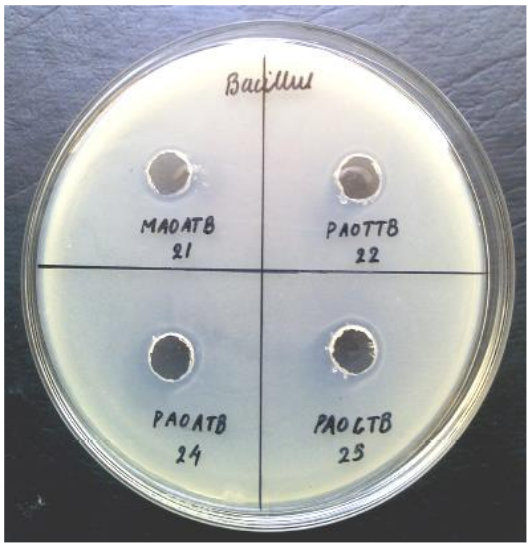

Plate-3 (3i, 3l, 3m)

Plate-4 (3o)

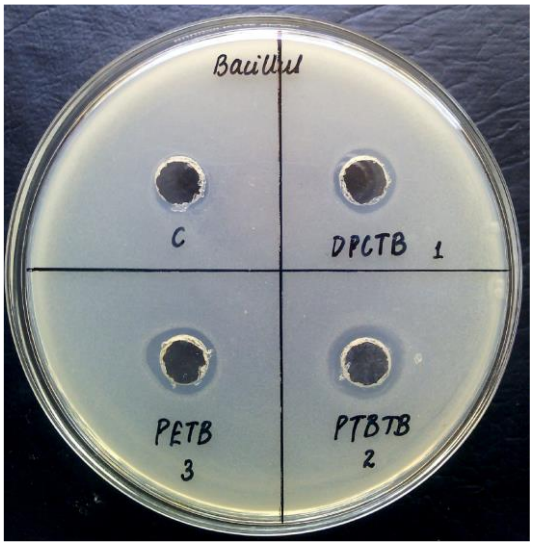

Plate-5 (DMSO)

Escherichia coli:

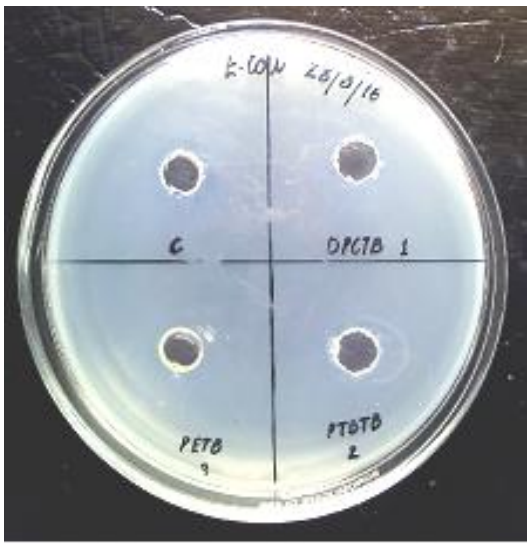

Plate-6 (DMSO)

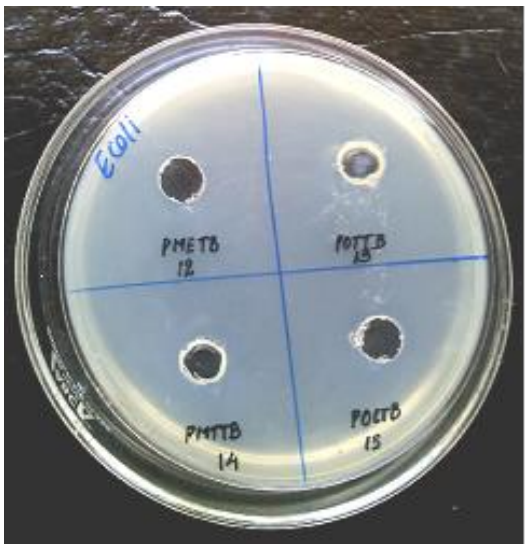

Plate-7 (3a, 3b 3c)

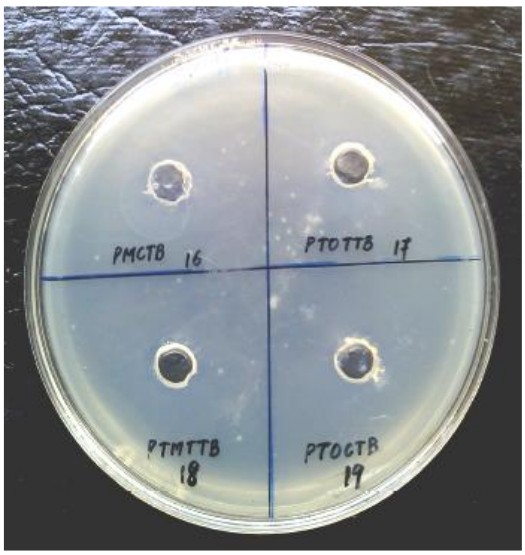

Plate-8 (3d, 3e, 3f, 3g)

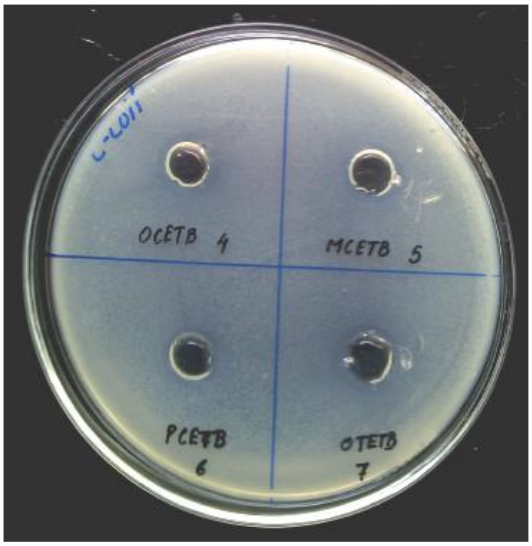

Plate-9 (3o)

Candida albicans:

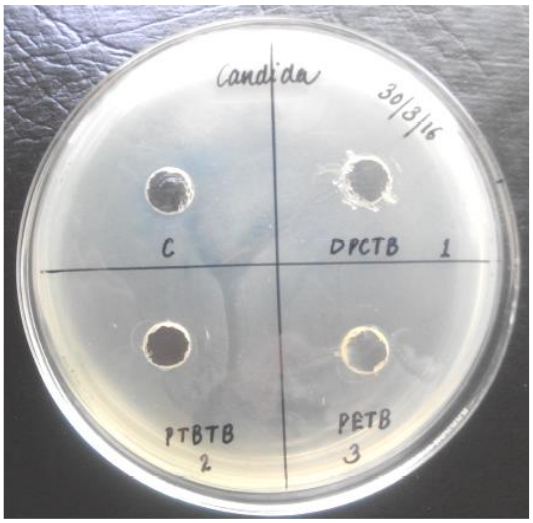

Plate-10 (DMSO)

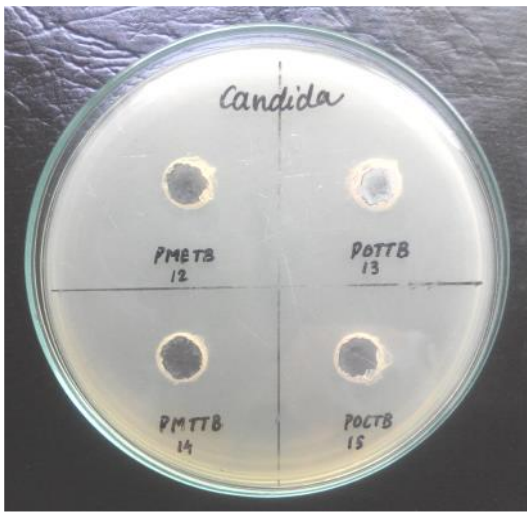

Plate-11 (3a, 3b, 3c)

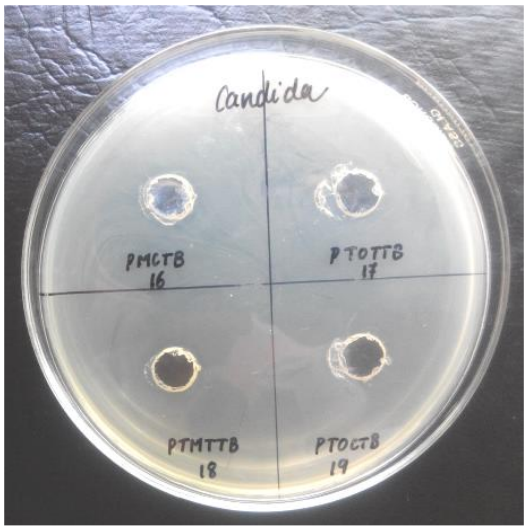

Plate-12 (3d, 3e, 3f, 3g)

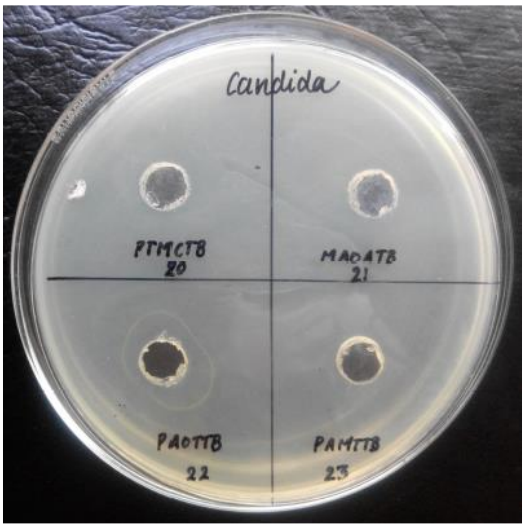

Plate-13 (3h, 3i, 3k)

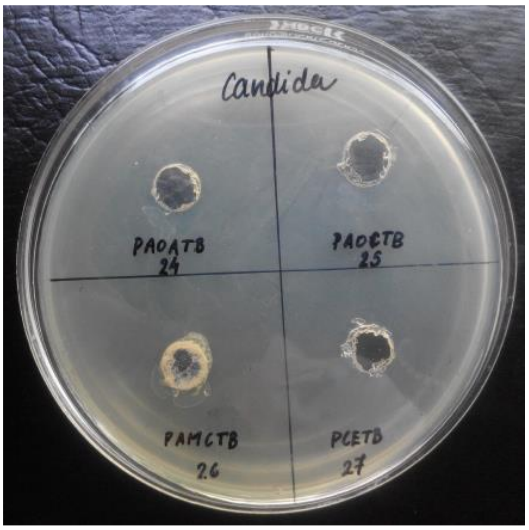

Plate-14 (3l, 3m, 3n, 3o)

Table 2. Name of thiobarbituric acids with their number and code

Compound No.	Code	Names of thiobarbituric acids
3a	POTTB	3-phenyl-2-thioxo-1-o-tolyl-dihydropyrimidine-4,6(1H, 5H)-dione
3b	PMTTB	3-phenyl-2-thioxo-1-m-tolyl-dihydropyrimidine-4,6(1H,5H)-dione
3c	POCTB	3-phenyl-2-thioxo-1-o-chlorophenyl-dihydropyrimidine-4,6(1H,5H)-dione
3d	PMCTB	3-phenyl-2-thioxo-1-m-chlorophenyl-dihydropyrimidine-4,6(1H,5H)-dione
3e	PTOTTB	3-o-tolyl-2-thioxo-1-p-tolyl-dihydropyrimidine-4,6(1H,5H)-dione
3f	PTMTTB	3-m-tolyl-2-thioxo-1-p-tolyl-dihydropyrimidine-4,6(1H,5H)-dione
3g	PTOCTB	1-p-tolyl-2-thioxo-3-o-chlorophenyl-dihydropyrimidine-4,6(1H,5H)-dione
3h	PTMCTB	1-p-tolyl-2-thioxo-3-m-chlorophenyl-dihydropyrimidine-4,6(1H,5H)-dione
3i	PAOTTB	3-o-tolyl-2-thioxo-1-p-anisole-dihydropyrimidine-4,6(1H,5H)-dione
3k	PAMTTB	3-m-tolyl-2-thioxo-1-p-anisole-dihydropyrimidine-4,6(1H,5H)-dione
3l	PAOATB	3-o-anisole-2-thioxo-1-p-anisole-dihydropyrimidine-4,6(1H,5H)-dione
3m	PAOCTB	3-o-chlorophenyl-2-thioxo-1-p-anisole-dihydro-pyrimidine-4,6(1H,5H)-dione
3n	PAMCTB	3-m-chlorophenyl-2-thioxo-1-p-anisole-dihydro-pyrimidine-4,6(1H,5H)-dione
3o	PCETB	1-p-chlorophenyl-2-thioxo-3-ethyl-dihydropyrimidine-4,6(1H,5H)-dione

Table 3. Antibacterial activity of Knoevenagel products against microorganisms

Compound No.	Code	Compound Code	Microorganisms			
			S. Aureus	**Plate No**	***E. coli***	**Plate No**
3b	K1	PPTAK	14	15	11	19
3l	K2	PPTCK	15	15	9	19
3p	K3	PTMTK	15	15	8	19
3u	K4	PTPAK	13	15	9	19
3x	K5	PTECK:	**18**	16	8	20
3t	K6	PCECK	16	16	8	20
3m	K7	PPACK	**17**	16	9	20
3c	K8	PPAAK	13	16	8	20
3e	K9	DMTAK	14	17	9	21
3g	K10	DMAAK	16	17	8	21
3a	K11	DPAK	15	17	9	21
3f	K12	DPTAK	14	17	8	21
3d	K13	DOTAK	15	18	8	22
3h	K14	DPAAK	12	18	8	22
3i	K15	DMCAK	**17**	18	8	22
3j	K16	DPCAK	13	18	8	22
Control	DMS		8		8	

(Concentration 100 µg/ml) (Inhibition zone in mm)

(--): Resistant (10 mm and less); sensitive (11-13 mm); Moderately sensitive (14-15 mm); Highly sensitive (16 mm and above)

Plates: Antimicrobial activity of Knoevenagel products against various microorganisms.

Staphylococcus aureus:

Plate-15 (3b, 3l, 3p, 3u)

Plate-16 (3c, 3m, 3t, 3x)

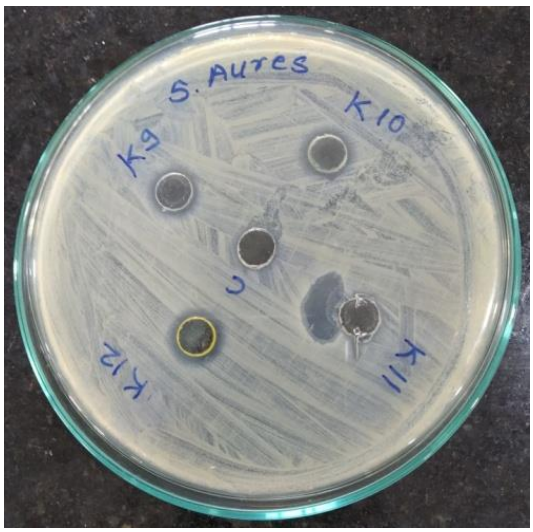

Plate-17 (3a, 3e, 3f, 3g)

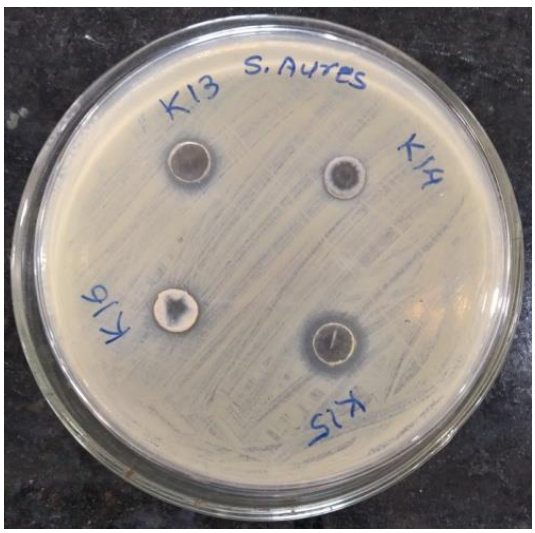

Plate-18 (3d, 3h, 3i, 3j)

***Escherichia coli*:**

Plate-19 (3b, 3l, 3p, 3u)

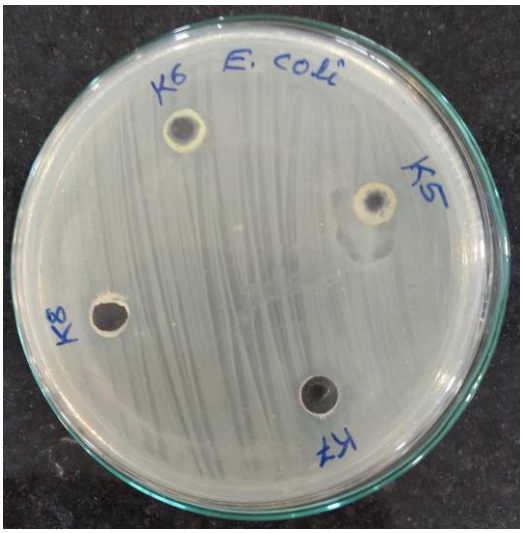

Plate-20 (3c, 3m, 3t, 3x)

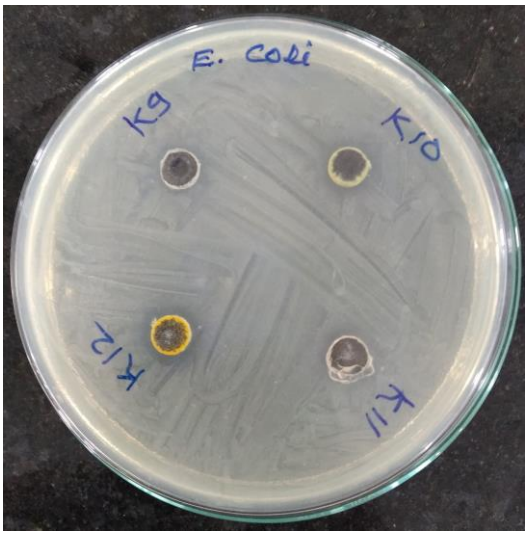

Plate-21 (3a, 3e, 3f, 3g)

Plate-22 (3d, 3h, 3i, 3j)

Table 4. Name of Knoevenagel products with their number and code

Compound No.	Code	Name of Knoevenagel products
3a	DPAK	5-(4-methoxybenzylidene)-1,3-diphenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3b	PPTAK	5-(4-methoxybenzylidene)-1-(4-methylphenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3c	PPAAK	5-(4-methoxybenzylidene)-1-(4-methoxylphenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(H,5H)-dione
3d	DOTAK	5-(4-methoxybenzylidene)-1,3-bis(2-methylphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3e	DMTAK	5-(4-methoxybenzylidene)-1,3-bis(3-methylphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3f	DPTAK	5-(4-methoxybenzylidene)-1,3-bis(4-methylphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3g	DMAAK	5-(4-methoxybenzylidene)-1,3-bis(3-methoxyphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3h	DPAAK	5-(4-methoxybenzylidene)-1,3-bis(4-methoxylphenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3i	DMCAK	1,3-bis(3-chlorophenyl)-5-(4-methoxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3j	DPCAK	1,3-bis(4-chlorophenyl)-5-(4-methoxybenzylidene)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3l	PPTCK	5-(4-chlorobenzylidene)-1-(4-methylphenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3m	PPACK	5-(4-chlorobenzylidene)-1-(4-methoxyphenyl)-3-phenyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3p	PTMTK	5-(4-chlorobenzylidene)-1-(3-methylphenyl)-3-(4-methyl-phenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3t	PCECK	5-(4-chlorobenzylidene)-1-(4-chlorophenyl)-3-ethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3u	PTPAK	5-(4-chlorobenzylidene)-1-(4-methoxyphenyl)-3-(4-methyl phenyl)-2-thioxodihydropyrimidine-4,6(1H,5H)-dione
3x	PTECK:	5-(4-chlorobenzylidene)-1-(4-methylphenyl)-3-ethyl-2-thioxodihydropyrimidine-4,6(1H,5H)-dione

Experimental

General

The present antimicrobial study deals with the antibacterial activity against Gram positive strain ***Bacillus cereus*** , ***Staphylococcus aureus*** and Gram negative strains, ***Escherichia coli*** and antifungal activity against ***Candida albicans*** using cup-plate agar diffusion method.

Antibacterial activity

The media used as Nutrient broth and his composition is Peptone (10 g), NaCl (10 g), Yeast extract (5 g), and Agar (20 g) in 1000 ml of distilled water. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37 ºC for 18 h. The agar plates of the above media were prepared and wells were made in the plate. For aseptic conditions, operations were carried out under ultra clean air laminar flow system. Each plate was inoculated with 18 h old cultures (100 µl, 10^4 CFU) and spread evenly on the plate. After 20 min, the wells were filled with samples. All the plates were incubated at 37 ºC for 24 h and the diameter of inhibition zones were noted.

Antifungal activity

The media used as Czapek-Dox Agar and his Composition is Sucrose (30 g), Sodium nitrate (2 g), K_2HPO_4 (1 g), $MgSO_47H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4$ (0.01 g) and Agar (20 g) in 1000 ml distilled water. Initially, the stock cultures were revived by inoculating in broth media and grown at 27 ºC for 48 h. The agar plates of the above media were prepared and wells were made in the plate. For aseptic conditions, operations were carried out under ultra clean air laminar flow system. Each plate was inoculated with 48 h old cultures (100 µl, 10^4 CFU) and spread evenly on the plate. After 20 min, the wells were filled with samples. All the plates were incubated at 27 ºC for 48 h and the diameter of inhibition zone were noted.

Experimental for title compounds

Test solutions were prepared with known weight of compound in dimethyl sulphoxide (DMSO) and diluted suitably to give the resultant concentration of 100 or 500 µg/mL and cups (wells) were loaded with 0.1 ml solution and plain DMSO solvent was used as a control. The media plates were incubated at 37 °C for 24 h (bacteria), 27 °C for 48 h (fungi). After the incubation inhibitory action of the test compounds were observed and a clear zone around the cups (wells) were measured as diameter expressed in millimeter (mm) and results were compared with standard antibacterial and antifungal drugs.

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