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Evaluation of Antimicrobial Effects of Different Substituted Arylpropeneones

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KEYWORDS

Solvent free Aldol condensation; 3-chloro-2-methylphenyl chalcones; Kirby baurer method; SAR study; Antimicrobial activities.

ABSTRACT:

A series containing nine substitutes styryl 3-chloro-2-methylphenyl ketones have been synthesised by fly-ash:H2SO4 catalysed solvent free Aldol condensation between 3-chloro-2-methyl acetophenone and substituted benzaldehydes. The yields are more than 90%. These chalcones were characterised by their physical constants and spectral data. d evaluated for its antimicrobial potential using the standardized Kirby-Bauer disc diffusion method. The compounds were screened for antibacterial activity against five bacterial strains (Micrococcus luteus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli) and for antifungal activity against two fungal species (Aspergillus niger, Penicillium spp.). ntibacterial screening against five bacterial strains revealed the 4-methyl derivative as the lead compound, showing the broadest spectrum and notable activity against Staphylococcus aureus. Antifungal testing against Aspergillus niger and Penicillium species demonstrated significantly higher potency, with the same 4-methyl analogue exhibiting superior, broad-spectrum efficacy, nearly matching the standard drug miconazole against Penicillium. The study establishes clear structure-activity relationships, highlighting the critical role of the substituent's electronic nature and position. Electron-donating groups, particularly at the para position, enhanced bioactivity, while meta- versus para- substitution often dictated striking species selectivity. These results identify the 4-methyl-substituted scaffold as a promising dual-activity lead for the development of novel antimicrobial agents.

1. Introduction

Chalcones (1,3-diaryl-2-propen-1-ones) represent a privileged scaffold in medicinal chemistry, serving as a fundamental precursor for numerous bioactive heterocycles and exhibiting a broad spectrum of pharmacological activities in their own right [1,2]. Their structural simplicity, featuring two aromatic rings linked by an α,β-unsaturated carbonyl system, allows for facile synthetic modification, making them an ideal template for structure-activity relationship (SAR) studies. This α,β-unsaturated ketone moiety is a critical pharmacophore, often associated with diverse biological properties, including antimicrobial [3], anticancer [4], anti-inflammatory [5], and antioxidant activities [6]. The pursuit of novel antimicrobial agents has intensified in response to the global crisis of multidrug-resistant pathogens. Chalcones have emerged as promising candidates in this arena due to their ability to interfere with key microbial targets, such as membrane integrity, enzymes like enoyl-acyl carrier protein reductase, and efflux pumps [7,8]. The Claisen-Schmidt condensation, typically between an aryl aldehyde and an aryl methyl ketone under basic or acidic catalysis, remains the most efficient and widely adopted method for chalcone synthesis. This reaction's versatility facilitates the strategic incorporation of diverse electron-donating and electron-withdrawing substituents, enabling systematic exploration of their electronic and steric effects on bioactivity [9,10].

Recent studies underscore the significant impact of halogen and alkyl substituents on the phenyl rings of chalcones. Halogenation, particularly with chlorine and bromine, is a classic bioisosteric strategy known to enhance lipophilicity, membrane permeability, and receptor binding, often boosting antimicrobial potency [11,12]. Concurrently, the introduction of methyl groups can modulate electron density and steric bulk, influencing interactions with biological targets [13,14]. A comprehensive analysis coupling synthesis with detailed spectral

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characterization (using FT-IR, NMR, and Mass spectrometry) and biological evaluation is essential to decode the underlying SAR and identify lead compounds. In this context, the present work details the synthesis of a novel series of substituted styryl 3-chloro-2-methylphenyl ketones or chalcones incorporating a fixed 3-chloro-2-methylphenyl ring and a variably substituted benzylidene moiety. The study employs a robust synthetic protocol, followed by meticulous spectral elucidation to confirm structures. The core objective is to evaluate their in vitro antibacterial and antifungal activities systematically, thereby investigating the influence of different para- and meta-substituents (H, Br, Cl, F, CH_3, NO_2) on antimicrobial efficacy. This integrated approach aims to contribute novel chemical entities to the antimicrobial discovery pipeline and furnish insightful SAR to guide the rational design of more potent chalcone-based therapeutics.

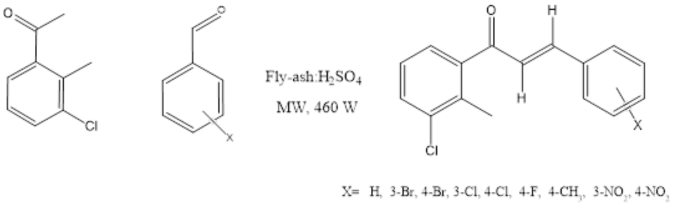
2. Materials and Methods

2.1. General

All chemicals used were purchased from Sigma-Aldrich and E-Merck chemical company. Melting points of all chalcones were determined in open glass capillaries on Mettler FP51 melting point apparatus and are uncorrected. The UV spectra of all synthesized chalcones were recorded in SHIMADZU-1650 SPECTROMETER (λ_{max} nm) in spectral grade methanol. Infrared spectra (KBr, 4000-400cm^{-1})were recorded on AVATAR-300 Fourier transform spectrophotometer. INSTRUM AV300 operating at 500MHz was used for ^{1}H spectra and 125.46MHz for ^{13}C spectra in DMSO solvent using TMS as internal standard. Mass spectra were recorded on a SIMADZU GC-MS2010 Spectrometer using Electron Impact (EI) techniques.

2.2. Synthesis of chalcones

An appropriate equi-molar quantities of 3-chloro-2-methylacetophenone (2 mmol), substituted benzaldehydes (2 mmol) and fly-ash:H_2SO_4 [15] (0.5 g) were taken in borosil tube and tightly capped. The mixture was subjected to microwave heated for 8-10 minutes in a microwave oven (**Scheme 1**) (LG Grill, Intellowave, Microwave Oven, 160-800W) and then cooled to room temperature. The organic layer was separated with dichloromethane and the solid product was obtained on evaporation. The solid, on recrystallization with benzene-hexane mixture gave glittering solid. The insoluble catalyst was recycled by washing the solid reagent remained on the filter by ethyl acetate (8 mL) followed by drying in an oven at 100°C for 1h and it was made reusable for further reactions.



Scheme 1. Synthesis of 3-chloro-2-methylphenyl chalcones.

3. Results and Discussion

Microbial activities

Chalcones possess a wide range of biological activities such as antibacterial, antifungal [16], antiviral [17], antifeedant [18], anticancer [19], antimalarial [20], antituberclosis [21], antiAIDS [22] and antioxidant [23] activities. These multipronged activities present in different chalcones are examined against respective microbes-bacteria's and fungi.

3.1. Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Kirby-Bauer [24] disc diffusion technique. In each Petri plate about 0.5 ml of the test bacterial sample was spread uniformly over the solidified Mueller Hinton agar using sterile glass spreader. Then the discs with 5mm diameter made up of Whatmann No.1 filter paper, impregnated with the solution of the compound were placed on the medium using sterile forceps. The plates were incubated for 24 hours at 37ºC by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24 hours, the plates were visually examined and the diameter values of the zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

The antibacterial screening effect of synthesized chalcones is shown in **Figure-1; (Plates 1-10)**. The zone of inhibition is compared using **Table-1** and the Clustered column Chart is shown in **Figure-2.**

Based on the biological screening data presented in Table-1, the synthesized series of substituted styryl 3-chloro-2-methylphenyl ketones demonstrates a clear and structurally dependent antibacterial profile, establishing a foundational structure-activity relationship (SAR). While none of the tested compounds surpass the broad-spectrum efficacy of the standard antibiotic ampicillin which exhibited robust inhibition zones between 10 and 14 mm across all bacterial strains that several derivatives emerged with noteworthy, selective activity, indicating their potential as leads for further development. The antibacterial effects were neither uniformly potent nor universally broad, but instead revealed significant variations contingent upon the electronic and steric properties of the substituent (X) and its precise position on the phenyl ring.

A critical analysis identifies the 4-methyl derivative (Entry 7) as the most well-rounded compound within the series. This electron-donating group conferred the broadest spectrum of activity, inhibiting all five tested organisms, and yielded the highest single activity measurement in the set, a 10 mm zone against Staphylococcus aureus, a clinically significant Gram-positive pathogen. Its consistent performance against both Gram-positive (*Micrococcus luteus, Bacillus subtilis, S. aureus*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria suggests favorable interactions with a bacterial target or enhanced cellular penetration. In stark contrast, the 4-fluoro derivative (Entry 6) presented a compelling case of selective potency, exhibiting its strongest effect against the often-treatment-resistant Gram-negative *Pseudomonas aeruginosa* (10 mm) while showing no activity whatsoever against Bacillus subtilis. This stark selectivity underscores how a subtle change, like the introduction of a small, highly electronegative atom at the para position, can critically alter the biological specificity, possibly by fine-tuning molecular interactions with disparate bacterial targets or uptake mechanisms.

Further dissection of the SAR reveals the paramount importance of substituent position. This is particularly evident with the nitro group: the 3-nitro isomer (Entry 8) maintained activity against all five strains, whereas the 4-nitro isomer (Entry 9) completely lost inhibitory power against *S. aureus*. A similar positional effect was observed with halogen atoms; the 4-chloro and 4-bromo compounds (Entries 5 & 3) were inactive against Micrococcus luteus, while their meta-substituted analogues (Entries 4 & 2) retained weak activity. The parent, unsubstituted compound (Entry 1) provided a consistent but weak baseline of activity (7-8 mm), confirming that the core scaffold possesses intrinsic antibacterial properties that can be modulated through synthetic modification.

The collective pharmacological profile of these compounds, characterized by generally modest inhibition zones (mostly below 10 mm), suggests that at the tested concentration, they likely function through a bacteriostatic mechanism that inhibiting bacterial growth and reproduction which rather than a directly lethal bactericidal action. The absence of a smooth correlation between activity and simple properties like hydrophobicity (as inferred from halogenated versus alkylated derivatives) argues against a non-specific mechanism such as generalized membrane disruption. Instead, the sharp dependence on substituent identity and orientation strongly implies a target-specific interaction, potentially involving the inhibition of a crucial bacterial enzyme or interference with a metabolic pathway where the methyl group enhances binding affinity or the fluorine atom creates a specific electronic complementarity.

3.2. Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer [24] disc diffusion technique. PDA medium was prepared and sterilized as above. It was poured (ear bearing heating condition) in the Petri-plate which was already filled with 1 ml of the fungal species. The plate was rotated clockwise and counter clock-wise for uniform spreading of the species. The discs were impregnated with the test solution. The test solution was prepared by dissolving 15mg of the chalcone in 1ml of DMSO solvent. The medium was allowed to solidify and kept for 24 hours. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeating the same procedure.

The antifungal activities of substituted chalcones synthesized in the present study are shown in **Figure-3** for **Plates (1-4)** and the zone of inhibition values of the effect is given in **Table-2**. The clustered column chart, shown in **Figure-4** reveals that all the compounds have excellent antifungal activity against all the two fungal species namely *A.niger* and *Penicilium scups*. The antifungal screening data for the series of substituted styryl 3-chloro-2-methyl ketones reveals a distinct and potent antifungal profile, demonstrating significant and highly substituent-dependent activity against two common filamentous fungi, Aspergillus niger and Penicillium species. Unlike the antibacterial results, several compounds in this series approach or, in one critical case, nearly match the potency of the standard antifungal drug miconazole, highlighting the promising potential of this chemical scaffold for antifungal development. The parent unsubstituted compound (Entry 1, X=H) itself establishes a strong baseline, exhibiting notable activity against *A. niger* (10 mm) and moderate activity against *Penicillium* (7 mm), indicating that the core structure is intrinsically bioactive against fungi.

The most outstanding performer in the series is unequivocally the 4-methyl derivative (Entry 7, X=4-CH_3). This compound demonstrated superior and broad-spectrum antifungal activity, producing an inhibition zone of 10 mm against A. niger and 11 mm against Penicillium. Its activity against Penicillium is particularly impressive, nearly rivaling the 14 mm zone of the standard miconazole. This consistent enhancement across both fungal strains, mirroring its strong antibacterial performance, underscores the 4-methyl group as a critically favorable modification for boosting bioactivity within this ketone series, likely by optimizing lipophilicity and target interaction. The analysis also uncovers a striking pattern of selective and often species-specific antifungal activity governed by the substituent's position. The 4-chloro derivative (Entry 5) was highly active against A. niger (8 mm) but completely inactive (-) against Penicillium. Conversely, the 3-bromo derivative (Entry 2) showed the opposite trend, being inactive against A. niger while retaining baseline activity (7 mm) against Penicillium. This dramatic selectivity suggests that different fungi may have varied uptake mechanisms, efflux systems, or target site sensitivities, which are differentially affected by the meta- versus para- positioning of halogen atoms. Other halogenated derivatives (3-Cl, 4-Br, 4-F) generally showed moderate, uniform activity against both fungi when active, with the 4-fluoro compound (Entry 6) showing consistent, if not exceptional, inhibition (7 mm against both).

Compounds bearing the strong electron-withdrawing nitro group (Entries 8 & 9, X=3-NO_2 & 4-NO_2) displayed weaker but measurable activity (6-8 mm), with slightly better performance against Penicillium than A. niger. Their activity, though present, is significantly lower than that of the methyl-substituted lead, suggesting that for antifungal action within this series, electron-donating or moderately hydrophobic groups are more beneficial than strongly electron-withdrawing ones.

4. Conclusions

We have synthesized a series of substituted styryl 3-chloro-2-methylphenyl ketones by microwave assisted Silica-H_2SO_4 catalyzed green Crossed-Aldol condensation of 3-chloro-2-methyl acetophenone and substituted benzaldehydes. These chalcones were characterized by their physical constants and spectral data. The antimicrobial activities of all chalcones have been studied using Bauer-Kirby method. The systematic evaluation of the synthesized substituted styryl 3-chloro-2-methylphenyl ketones via standardized disc diffusion assays has successfully established their biological potential, revealing distinct and promising antimicrobial profiles. The investigation delineates clear structure-activity relationships (SAR) critical for future development. In the antibacterial sensitivity assay, the series demonstrated modest to moderate activity against a panel of Gram-positive and Gram-negative bacteria. While none of the derivatives surpassed the standard antibiotic ampicillin, the 4-methyl analogue (Entry 7) emerged as the lead antibacterial candidate, exhibiting the broadest spectrum of inhibition and the highest single activity (10 mm against Staphylococcus aureus). The assays further highlighted the critical influence of substituent position and electronic nature, as evidenced by the selective anti-pseudomonal activity of the 4-fluoro derivative and the differential effects of meta- versus para- halogen and nitro substituents. The general

profile suggests a bacteriostatic, target-specific mechanism of action rather than non-specific membrane disruption, providing a focused hypothesis for mechanistic studies. Conversely, the antifungal sensitivity assay revealed a more potent and encouraging profile against Aspergillus niger and Penicillium species. Here, the same 4-methyl derivative (Entry 7) displayed exceptional, broad-spectrum efficacy, with activity against Penicillium (11 mm) approaching that of the standard drug miconazole (14 mm). The antifungal results also unveiled striking species-selective activity dependent on substituent position, such as the inactivity of the 4-chloro compound against Penicillium and the 3-bromo compound against A. niger. This selectivity underscores the scaffold's sensitivity to fungal biological differences and hints at potential for designing narrow-spectrum agents.

In summary, the antimicrobial screening conclusively identifies the 4-methyl substituted derivative as a dual-activity lead compound, warranting further investigation. The foundational SAR principles elucidated—highlighting the superiority of electron-donating groups and the profound impact of regiochemistry—provide a robust, actionable blueprint for the rational design and synthesis of next-generation analogues with optimized potency and spectrum for potential therapeutic applications.

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Table 1. Antibacterial activity of substituted styryl 3-chloro-2-methylphenyl ketones

Entry	X	Zone of Inhibition (mm)				
		Gram positive Bacteria			Gram negative Bacteria	
		Micrococcus	*B.subtilis*	*S.aureus*	*Pseudomonas*	*E.coli*
1	H	7	8	7	8	7
2	3-Br	6	7	8	7	8
3	4-Br	-	6	8	7	7
4		7	6	7	7	7

	3-Cl					
5	4-Cl	-5	7	7	8	8
6	4-F	8	-	9	10	7
7	4-CH_3	7	9	10	9	6
8	3-NO_2	7	7	8	9	8
9	4-NO_2	7	6	-	6	8
Standard	Ampicillin	10	11	13	12	14
Control	DMSO					

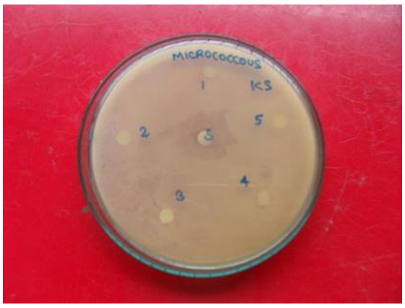


PLATE-1

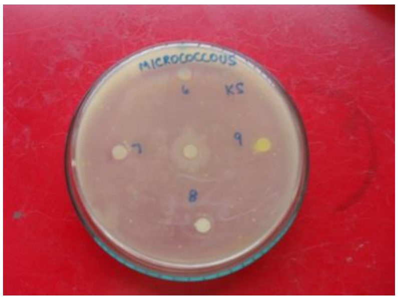
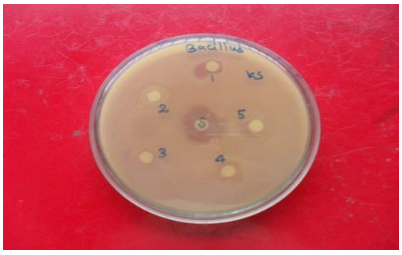


PLATE-2



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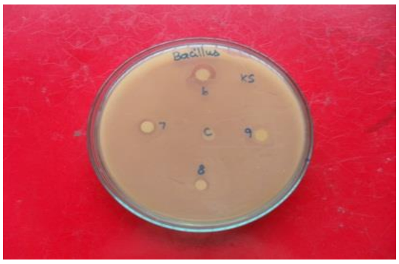
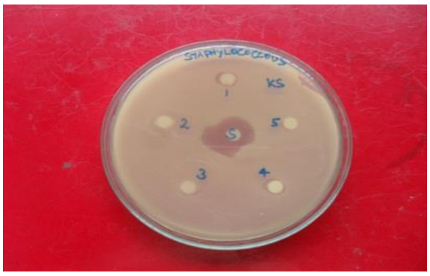


PLATE-4



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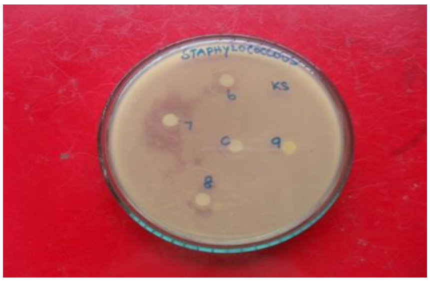

PLATE-6

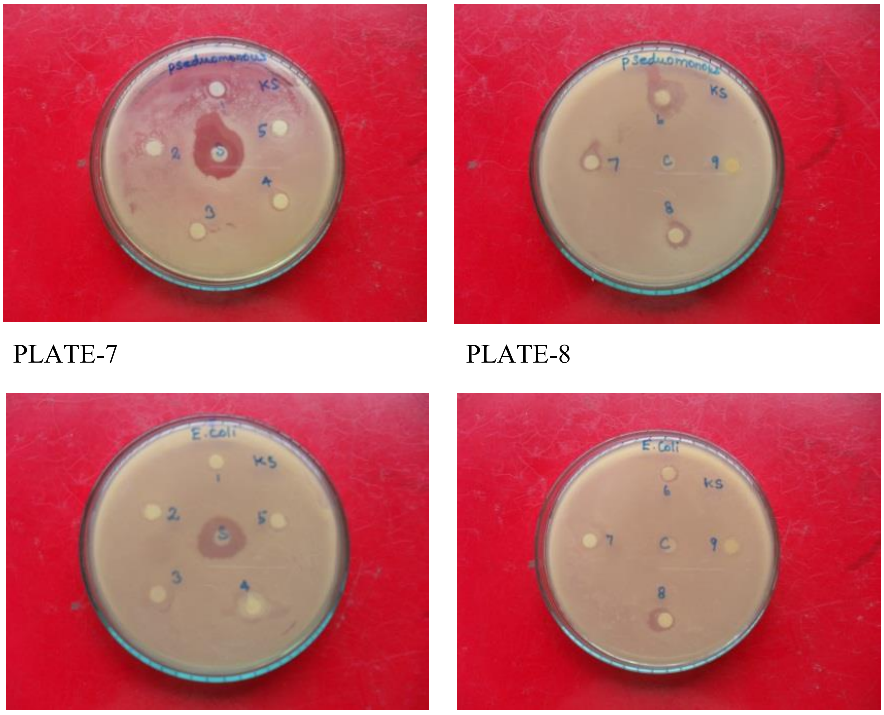

PLATE-7 PLATE-8

PLATE-9 PLATE-10

Figure-1. Antibacterial activity of styryl 3-chloro-2-methylphenyl ketones-petri dishes

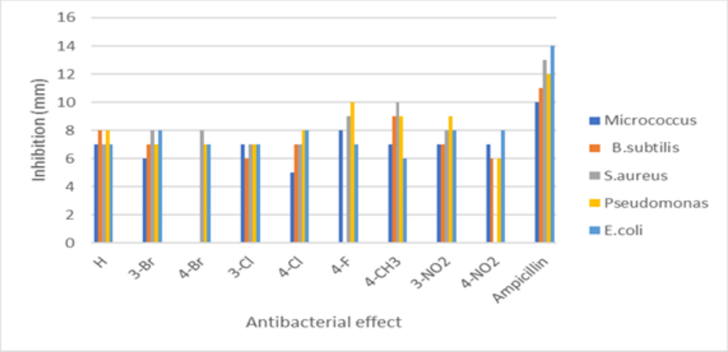

Figure-2. Antibacterial activity of styryl 3-chloro-2-methylphenyl ketones-Clustered column chart.

Entry	X	Zone of Inhibition (mm)	
		A.niger	*Penicilium Scup*
1	H	10	7
2	3-Br	-	7
3	4-Br	7	7
4	3-Cl	7	6
5		8	-

	4-Cl		
6	4-F	7	7
7	4-CH_3	10	11
8	3-NO_2	6	8
9	4-NO_2	6	8
Standard	Miconazole	13	14
Control	DMSO		

Table-2. Antifungal activities of substituted styryl 3-chloro-2-methylketones

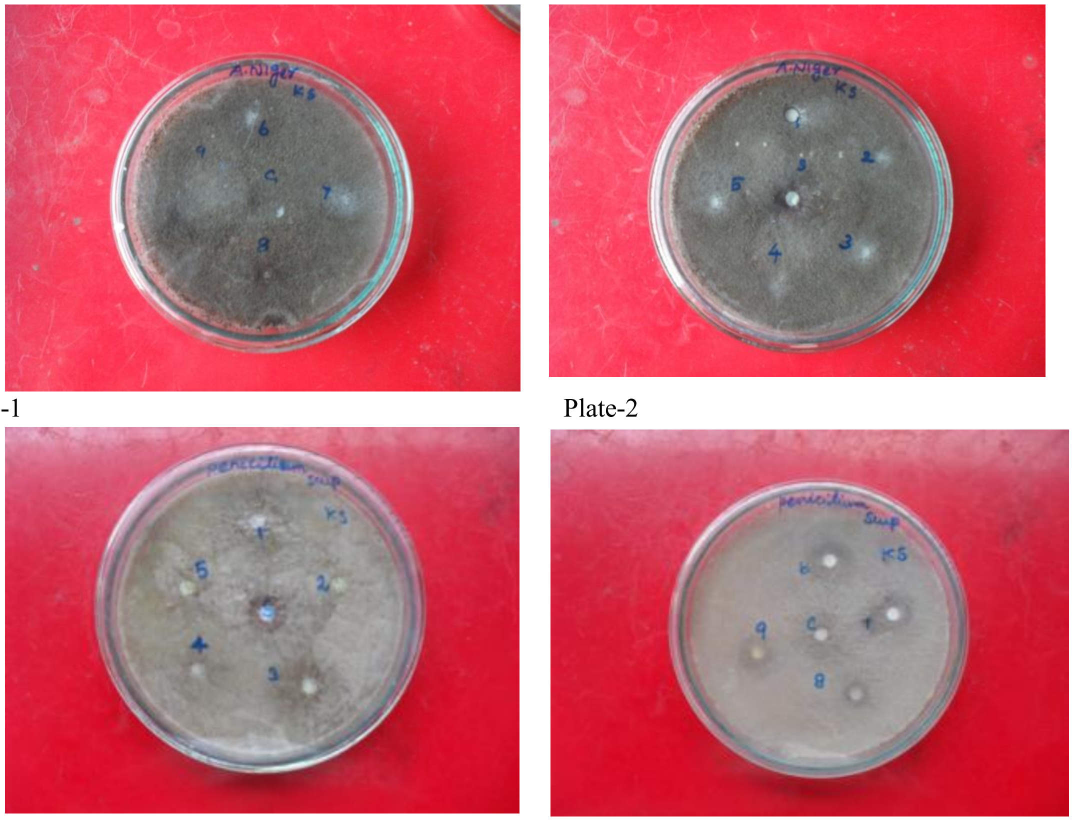

Plate-1 Plate-2

Plate-3 Plate-4

Figure-3. Antifungal activity of styryl 3-chloro-2-methylphenyl ketones-petri dishes.

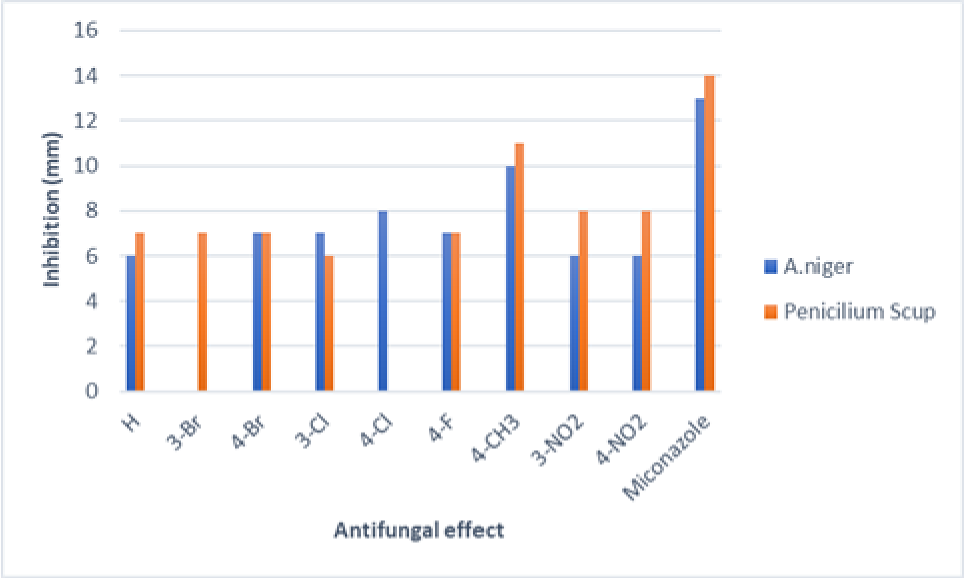

Figure-4. Antifungal activity of styryl 3-chloro-2-methylphenyl ketones-Clustered column chart.