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Antimicrobial and antioxidant potent substituted styryl β-hydroxy-α-naphthyl ketones

Ganesamoorthy Thirunarayanan[1], Ganesan Vanangamudi[2], Muthuvel Subramanian[2]*

[1]Department of Chemistry, Annamalai University, Annamalainagar-608002, (INDIA)

[2]P G & Research Department of Chemistry, Government Arts College, C-Mutlur, Chidambaram-608102, (INDIA)

ABSTRACT

A series of twelve β-hydroxy-α-naphthyl chalcones [2*E*-1-(2-hydroxy-1-naphthyl)-3-(substituted phenyl)-2-propen-1-ones] were synthesized by Crossed-Aldol condensation of 1-acetyl-2-hydroxy naphthalene and substituted benzaldehydes. The purities of these chalcones have been checked by their physical constants and spectral data. The antimicrobial and antioxidant activities of these β-hydroxy-α-naphthyl 2-propen-1-ones have been studied using Bauer-Kirby and DPPH radical scavenging methods respectively.

KEYWORDS

β-hydroxy-α-naphthyl chalcones; Crossed-Aldol condensation; Antimicrobial and antioxidant activities.

INTRODUCTION

Chalcones are α, β-unsaturated ketones and they belongs to biomolecules. Many alkyl-alkyl, alkyl-aryl and aryl-aryl categories of chalcones were synthesized by solvent assisted[1] or solvent free methods[2-7] and extracted from natural plants[8] by organic chemists. Various methods available for synthesizing chalcones such as Aldol, Crossed-Aldol, Claisen-Schmidt, Knovenagal, Greener methods- Grinding of reactants, solvent free and oxides of nanoparticle with microwave heating. Due to C-C single bond rotation[9] of carbonyl and alkene carbons, they exist as *E s-cis* and *s-trans* and *Z s-cis* and *s-trans* conformers. This structural conformers of chalcones were confirmed by ^{1}H NMR and IR spectroscopy. These chalcones possess various multipronged activities[10]. Keto, alkene and the polar substituents in aryl or styryl phenyl moieties in the chalcones are responsible for their biological activities. The various biological activities of chalcones are antibacterial[11], antifungal[12], antioxidant[13], antiviral[14], antimalarial[15], anticancer[16], antiplosmodial[17], antituberclosis[18], antiproliferative[19,20], antileshmanial[21], anti-inflammatory[22], antianalgesic and sedative[23], radical scavenging[24], antitumour[25,26], antiperasitic[27], cytotoxicity[28], HIV-antiAIDS[29], cardiovasculant[30], inhibition of lipid peroxidation[31] and insect antifeedants[32,33]. Hydroxy chalcones possess more antioxidant activities. Halogenated chalcones possess insect antifeedant activities[32,33]. In the present study, the authors wishes to report solvent free synthesis, medicinal activities such as antimicrobial and antioxidants of some substituted styryl 2-hydroxy-1-naphthyl ketones.

EXPERIMENTAL

Materials and methods

All chemicals used were purchased from Sigma-Aldrich and E-Merck chemical companies. Melting points of all chalcones have been determined in open

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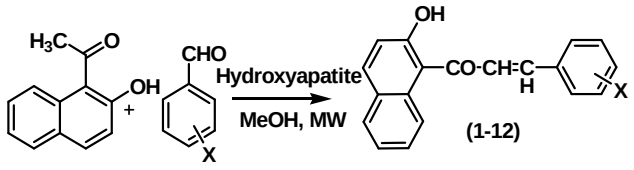
glass capillaries on Mettler FP51 melting point apparatus and are uncorrected. Infrared spectra (KBr, 4000-400 cm^{-1}) have been recorded on AVATAR-300 Fourier transform spectrophotometer. The NMR spectra of chalcones were recorded in INSTRUM AV300 NMR spectrometer operating at 300 MHz for ^{1}H and 75.46 MHz for ^{13}C spectra in DMSO solvent using TMS as internal standard. Electron impact (EI) (70 eV) and chemical ionization mode FAB^{+} mass spectra were recorded with a JEOL JMS600H spectrometer.

Synthesis of substituted styryl 2-hydroxy-1-naphthyl ketones

An appropriate equal molar quantity of 2-hydroxy-1-acetylnaphthalene (0.01mol), various substituted benzaldehydes (0.01mol), in methanol (10 mL) and 1 g hydroxyapatite were added and the mixture was stirred at room temperature for 5 min[34]. Methanol was evaporated to give a homogeneous solid. About 5 mL of water was added to this solid and the mixture was irradiated by microwave for the appropriate time (Scheme 1). After completion of reaction, dichloromethane (20 mL) was added, followed by simple filtration. The solution was concentrated and to purified by re-crystallization. The synthesized chalcones are characterized by their physical constants, IR, ^{1}H and ^{13}C NMR and Mass spectral data. Analytical and Mass spectral data are presented in TABLE 1.



X= H, 3-Br, 4-Br, 2-Cl, 3-Cl, 4-Cl, 2-OH, 4-OH,2-OCH_3, 4-CH_3, 2-NO_2, 3-NO_2

Scheme 1: Synthesis of substituted styryl β-hydroxy-α-naphthyl ketones

TABLE 1 : The physical constants, analytical and mass fragments (m/z) data of substituted styryl β-hydroxy-α-naphthyl ketones

Entry	X	Mol. Formula	Mol. Wt.	m.p. (°C)	Found (Calcd) (%)			MS Fragmentas (m/z)
					C	H	N	
1	H	$C_{19}H_{14}O_2$	274	101-102 (100-101)[25]	---	---	---	274[M^+]
2	3-Br	$C_{19}H_{13}BrO_2$	352	110-111 (111-112)[25]	---	---	---	352[M^+], 354[M^{+2}]
3	4-Br	$C_{19}H_{13}BrO_2$	352	106-107	64.59 (64.61	3.68 3.71)	---	352[M^+], 354[M^{+2}], 335, 208, 197, 180, 171, 154, 143, 79, 55, 17
4	2-Cl	$C_{19}H_{13}ClO_2$	308	112-13 (113-114)[25]	---	---	---	308[M^+], 310[M^{+2}]
5	3-Cl	$C_{19}H_{13}ClO_2$	308	102-103 (103-104)[25]	----	---	---	308[M^+], 310[M^{+2}], 291, 273, 197, 171, 165, 143, 137, 111, 77, 35, 17
6	4-Cl	$C_{19}H_{13}ClO_2$	308	86-87 (87-88)[25]	---	---	---	308[M^+], 310[M^{+2}], 273, 197, 171, 165, 143, 137,111, 77, 55, 17
7	2-OH	$C_{19}H_{14}O_3$	290	73-74 (75-76)[25]	---	---	---	290[M^+]
8	4-OH	$C_{19}H_{14}O_3$	290	71-72 (72-73)[25]	---	---	---	290[M^+], 273, 197, 171, 147, 143, 93, 77, 17
9	2-OCH_3	$C_{20}H_{16}O_3$	304	81-82 (82-83)[25]	---	---	---	304[M^+], 287, 273, 197, 171, 161, 143, 107, 91, 77, 55, 17
10	4-CH_3	$C_{20}H_{16}O_2$	288	97-98 (98-99)[25]	---	---	---	288[M^+]
11	2-NO_2	$C_{19}H_{13}NO_4$	319	116-17	71.52 (71.47	4.06 4.10	4.42 4.39)	319[M^+], 302, 273, 197, 176, 171, 143, 122, 45, 17
12	3-NO_2	$C_{19}H_{13}NO_4$	319	126-127 (125-126)[25]	---	---	---	319[M^+], 302, 273, 197, 176, 143, 122, 55, 77, 45, 17

Measurement of antimicrobial activities

Materials and methods

The chemicals namely nutrient broth, Mueller Hinton agar, potato dextrose agar, Tween-80 solution and other materials required have been procured from Himedia, Mumbai

Collection of microorganisms

Bacillus subtilis, Escherichia coli, Klebsila pneumonia, Micrococcus luteus, Pseudomonas aerogenosa, Staphylococcus areus, Aspergillus niger, Mucor species and *Trichoderma viride* were procured from the Research department of Microbiology, Sengunthar Arts and Science College, Thiruchengode,

Namakkal Dt., Tamilnadu.

Innoculum preparation

The nutrient broth was procured from Himedia, Mumbai. The nutrient broth was prepared by weighing 1.3 g, of the broth and dissolved it in 100 mL of sterile distilled water. The flask was swirled gently while adding the nutrient broth and the pH of the medium was adjusted to 7.0. The Erleumayer flask was plugged with non-adsorbent cotton and sterilized in an autoclave at 121°C and 15 lbs/inc^2 pressure for 15min. After cooling inside a laminar flow, a loopful of fresh bacterial sample was inoculated and incubated in an orbital shaker at 37°C for 24h. Then the cultures were diluted 1:50 with sterile physiological saline and 0.5 mL of the innoculum was used for the preparation of the spread plate. The same procedure has been adopted for all test bacterial samples.

Preparation of agar slants

Nutrients agar medium was prepared and sterilized in an autoclave at 121°C and 15 lbs/inc^2 pressure for 15 minutes. After sterilization the medium was dispensed into the test tubes. The test tubes were kept in the slanting position on a support. After complete solidification of the medium, streaking of the microorganism was done in the slant area using sterile inoculation loop. After the streaking the test tubes were incubated at 37°C for 24 h. After good growth, the slants have been stored in a deep freezer (2°C) for further studies.

Preparation of Mueller Hinton agar plates

The Mueller Hinton agar of weight 38 g was dissolved in 1000 mL of sterile distilled water. The pH of the medium was adjusted to 7.0. The flask was plugged with cotton and sterilized at 121°C and 15 lbs/inc^2 pressure for 15min. After sterilization, the medium was cooled to 45-47°C, poured 15 mL of it in each sterile Petri-plates and allowed to solidify.

Preparation of test compound

The synthesized chalcone compounds of weight 15 mg of each was dissolved in 1 mL of DMSO solvent. Using 100 µmL solution, the discs were impregnated and placed on the Mueller Hinton solidified Agar medium to find out the antimicrobial activity of the compounds on each organism.

Antibacterial sensitivity assay

Antibacterial sensitivity assay was performed using Kirby-Bauer[29] 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 5 mm diameter made up of Whatman No.1 filter paper, impregnated with the solution of the compound were placed on the medium using sterile foreceps. The plates were incubated for 24 h at 37°C by keeping the plates upside down to prevent the collection of water droplets over the medium. After 24h, 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.

Antifungal activity

Preparation of the potato dextrose agar medium

PDA agar medium was prepared in a conical flask by dissolving 3.9g of the agar in 100mL distilled water. It was sterilized in the autoclave for 15min. at 121°C and 15 lbs/inch^2 pressure. Then the medium was allowed for solidification for an hour. After that the fungal species was inoculated in the medium and kept for 5 to 7 days at room temperature.

Preparation of the fungal innoculam

About 20 to 25 mL of sterile water (after cooling) is mixed with the medium. The water over the medium is swirled and decanted with the fungal species. Tweeen-80(1 to 2 mL) may be added with this solution for uniform growth.

Antifungal sensitivity assay

Antifungal sensitivity assay was performed using Kirby-Bauer[29] 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 h. Then the plates were visually examined and the diameter values of zone of inhibition were measured. Triplicate results were recorded by repeat-

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ing the same procedure.

RESULTS AND DISCUSSION

The antibacterial and antifungal activities have been studied using bacterial and fungi strains. The gram positive bacterial strains are *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*. The gram negative bacterial strains are *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The fungal strains used in the present study are *Aspergillus niger*, *Mucor species* and *Trichoderma viride*. The antimicrobial activites of these hydroxyl naphthyl chalcones are screened using Bauer-Kirby[35] petri-dish method.

TABLE 2 : Antibacterial activity of substituted styryl β-hydroxy-α-naphthyl ketones

Entry	Compound	X	Zone of inhibition (mm)					
			Gram positive bacteria			Gram positive bacteria		
			B.subtilis	*M.luteus*	*S.aureus*	*E.coli*	*K.pneumoniae*	*P.aeruginosa*
1	A1	H	6	6	8	6	7	7
2	A2	3-Br	7	7	8	7	6	7
3	A3	4-Br	7	8	7	---	---	7
4	A4	2-Cl	7	10	7	10	7	8
5	A5	3-Cl	7	6	7	10	---	7
6	A6	4-Cl	7	10	8	10	6	6
7	A7	2-OH	6	6	---	8	8	7
8	A8	4-OH	---	8	10	7	---	8
9	A9	2-OCH_3	6	6	6	6	6	7
10	A10	4-CH_3	6	6	---	7	6	7
11	A11	2-NO_2	10	7	6	7	---	6
12	A12	3-NO_2	8	8	7	6	6	8
	Standard	Ampicillin	25	20	---	8	20	8
	Control	DMSO	---	---	---	---	---	---

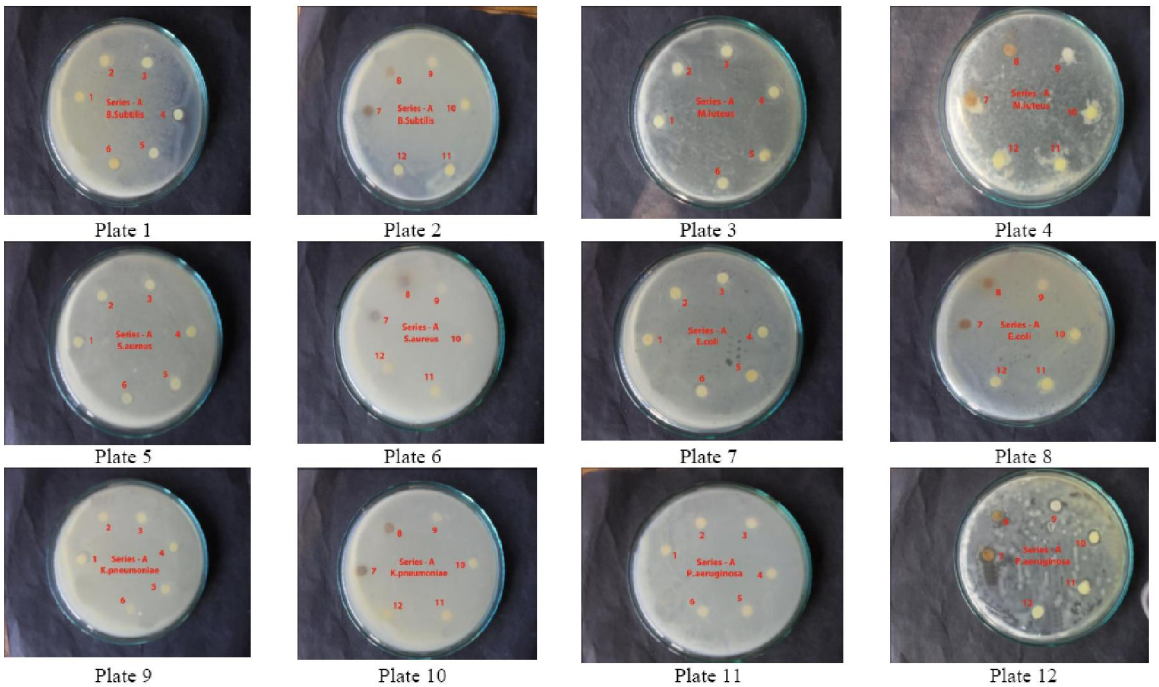

Figure 1: Antibacterial activities of substituted styryl β-hydroxy-α-naphthyl ketones-Petri-dish plates

The antibacterial effect of the substituted styryl 2-hydroxy-1-naphthyl ketones shows good antibacterial activity on all the six microorganism and are shown in Figure 1 (plates 1-12). On analyzing the zone of inhibition as given in TABLE 2. And the clustered column chart is shown in Figure 2. The chalcones with chloro substituents in 2, 3 and 4th positions in styryl phenyl moieties showed in excellent activities on *Micrococcus luteus*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. The chalcones with hydroxyl group in styryl moiety showed very good activity on *Staphylococcus aureus*.

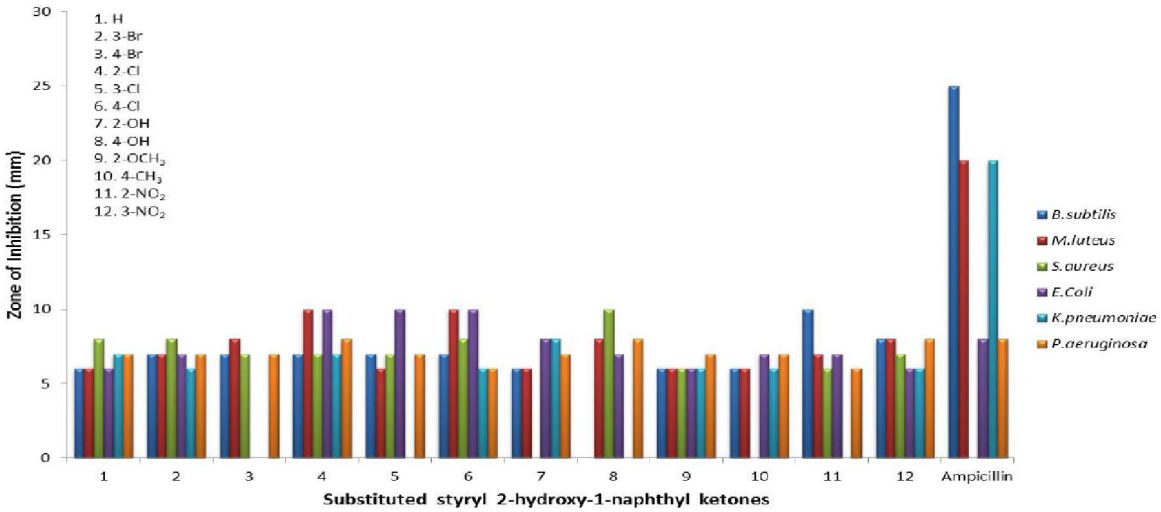

Figure 2 : Antibacterial activities of substituted styryl β-hydroxy-α-naphthyl ketones-Clustered column chart

The antifungal activities of 2-hydroxy-1-naphthyl chalcones have been screened using petri-dish technique are shown in Figure 3 (plates1-6), the zone of inhibition values are given in TABLE 3 and the clustered column chart is shown in Figure 4. The chalcones having halogens and nitro substituents in styryl phenyl moiety showed satisfactory effects of zone of inhibitions with *Aspergillus niger, Mucor species*.

TABLE 3 : Antifungal activity of substituted styryl β-hydroxy-α-naphthyl ketones

Entry	Compound	X	Zone of inhibition (mm)		
			A.niger	*M.spp*	*T.viride*
1	A1	H	--	7	---
2	A2	3-Br	5	7	---
3	A3	4-Br	---	6	---
4	A4	2-Cl	---	8	---
5	A5	3-Cl	---	---	---
6	A6	4-Cl	6	5	---
7	A7	2-OH	---	---	---
8	A8	4-OH	---	---	---
9	A9	$2\text{-}OCH_3$	---	6	---
10	A10	$4\text{-}CH_3$	---	---	6
11	A11	$2\text{-}NO_2$	---	---	---
12	A12	$3\text{-}NO_2$	6	8	8
	Standard	Miconazole	8	9	15

The antioxidant activities of all synthesized chalcones have been evaluated by the DPPH radical scavenging effect[36]. The 0.1 M acetate was prepared by dissolving 1.64 g of sodium acetate in 15 mL of water and 150 µL of acetic acid. The final volume was adjusted to 20 mL by adding water. The 0.2 mmol of DPPH solution was prepared by dissolving 3.9 g of DPPH in 50 mL of ethanol. ∝-Tocopherrol (1 mg in 10 mL of ethanol) solution was prepared. A series of test tubes were arranged with 1.0 mL of buffer solution mixed with 0.5 mL of DPPH solution. A series of various concentrations of synthesized chalcones and ∝-Tocopherol (1 µg in 1 mL of ethanol) were added to each tube and mixed well. After 30 min at RT the absorbance of each solution is measured by UV-Vis spectrophotometer at 517 nm. A mixture of buffer solution and ethanol were used as the reference for the spectrophotometer. A graph was plotted with the weight of the compound vs absorptions and IC_{50} values were determined. The antioxidant activity was expressed in terms of IC_{50} (µg/mL, concentration required to inhibit DPPH radical formation by 50%). ∝-Tocopherol was used as a positive control. From this experiment, hydroxyl and methoxy substituted chalcones were found to have a significant antioxidant activity.

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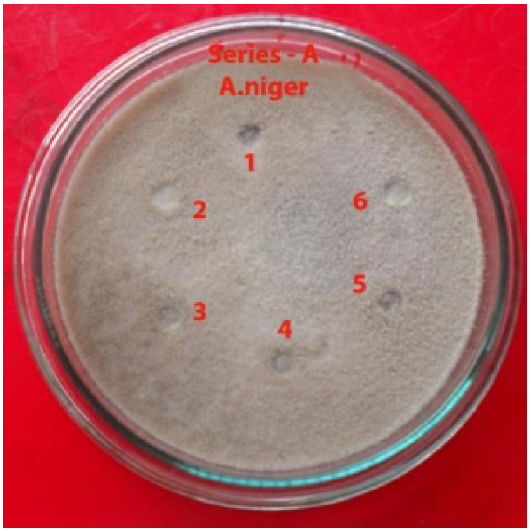
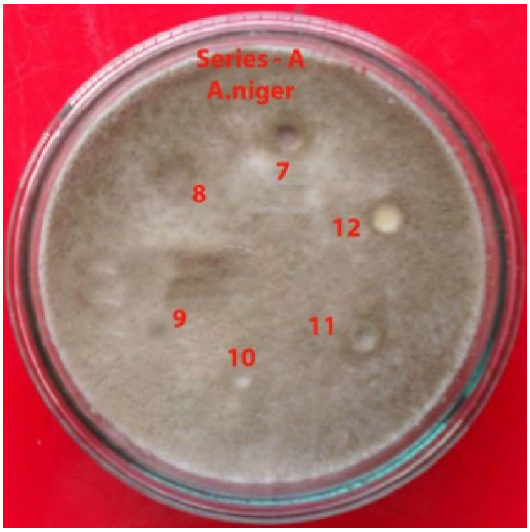


Plate 1



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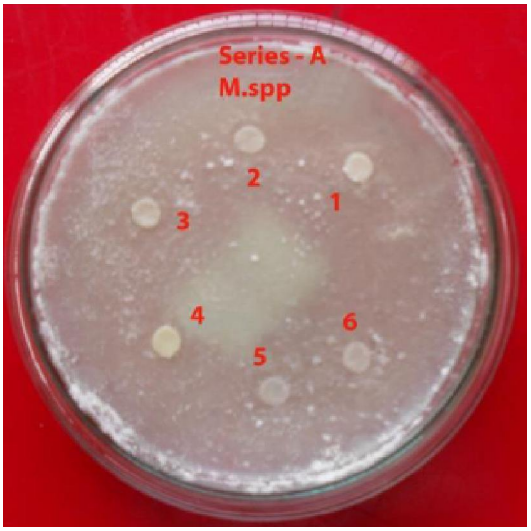

Plate 3

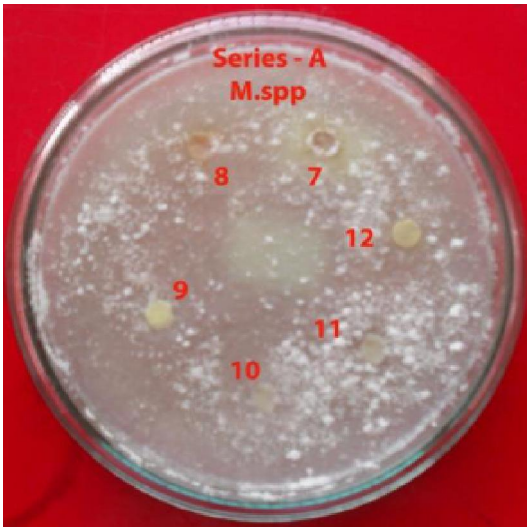

Plate 4

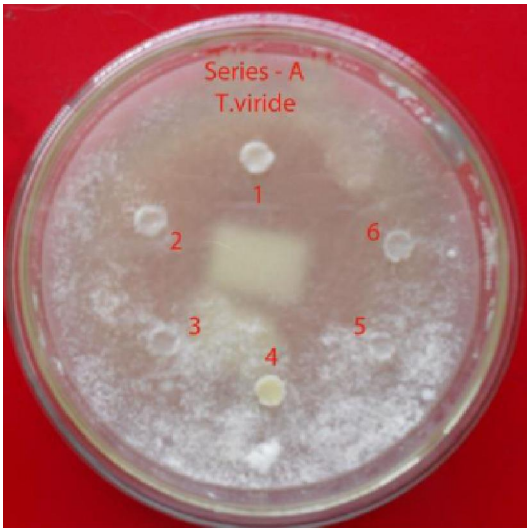

Plate 5

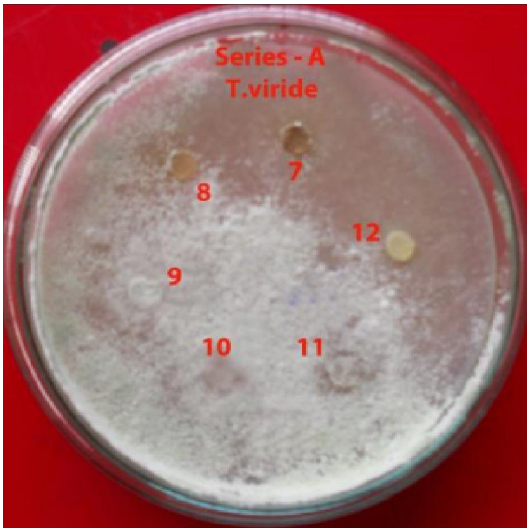

Plate 6

Figure 3 : Antifungal activities of substituted styryl β-hydroxy-α-naphthyl ketones-Petri-dish plates

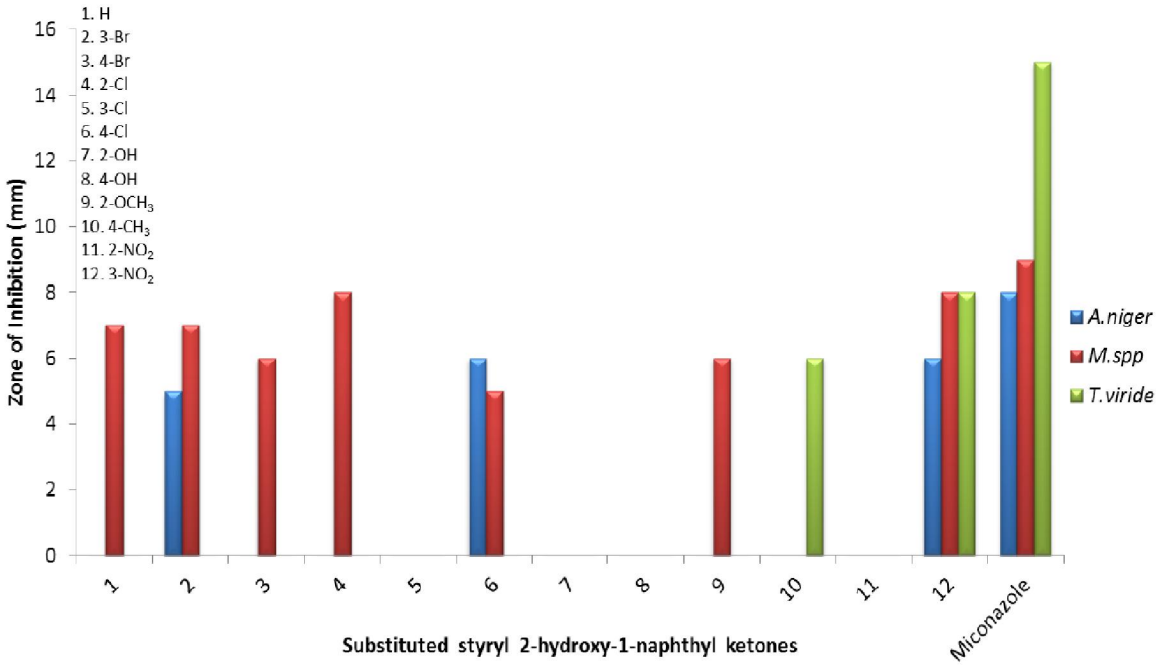

Figure 4 : Antifungal activities of substituted styryl β-hydroxy-α-naphthyl ketones-Clustered column chart

CONCLUSION

In conclusion, the authors have synthesized some substituted styryl 2-hydroxy-1-naphthyl ketones by crossed-aldol condensation of 2-hydrox-1-acetyl naphthalene and substituted benzaldehydes. The anti-microbial and antioxidant activities of these chalcones have measured by Bauer-Kirby and DPPH radical scavenging methods. The chalcones with chloro and hydroxy substituted chalcones shows good anti-microbial activities. The hydroxyl and methoxy substituted chalcones were found to have a significant antioxidant activity.

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