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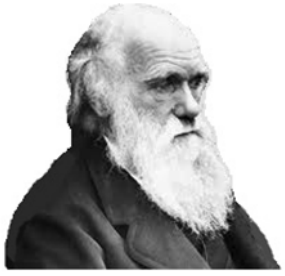

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Synthesis, characterization and antifungal activity of some fluorine containing 1,3,5-trisubstituted pyrazoline derivatives

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ABSTRACT

A series of (E)-1-(4-fluorophenyl)-3-substitutedphenylprop-2-en-1-one (3-7) were ultrasonically prepared by the reaction of 4-fluoroacetophenone with different aromatic aldehydes in the presence of alkali . Reaction of the prepared chalcones (3-7) with 3,4,5-trimethoxybenzohydrazide (8) afforded the corresponding substituted pyrazoline (9-13). Ultrasonic irradiation method provides several advantages over current reaction methodologies, including a simple work-up procedure, shorter reaction times and good yields. All the prepared compounds have been characterized by FT-IR and ^{1}H-NMR spectra. These compounds were screened for their antifungal activity using disc diffusion method. Compound 10 and 11 was found to exhibit the most potent in-vitro anti-fungal activity with against all the fungal strains.

***Keywords*:** chalcone, pyrazoline, ultrasonic irradiation, antifungal activity, disc diffusion method

1. INTRODUCTION

Discovery of novel synthetic heterocyclic compounds are the target of organic scientists to cure the diseases. Fluorine atom acting a significant role in the field of chemical and biochemical sciences. The literature review exposed that after nitrogen; fluorine occupies the position of the second most hetero element in the field of life science oriented research. Intrinsic properties of the fluorine atom, such as high electronegativity, small atomic radius, and low polarisability of the C–F bond, impart significant improvement on the biological

activity of fluorinated molecules [1]. Fluorinated compounds have proved invaluable as antibacterial and antifungal agents, and have been used for the treatment of obesity and various diseases associated with the cardiovascular and central nervous systems [2].

Chalcones are natural compounds found in various plants or they are synthetically synthesized. These substances are of a high attention due to their uses as preliminary compounds in the synthesis of a number of heterocyclic compounds [3]. Chalcones are unsaturated compounds that are major intermediates in the synthesis of natural products [4]. They are known to have various biological activities resembling fungicidal [5] properties. Among a wide range of heterocyclic compounds that have been explored for the development of pharmaceutically important molecules, Pyrazolines have proved their worth in the field of medicinal chemistry. Pyrazoles and their reduced forms, pyrazolines, are well known nitrogen containing heterocyclic compounds, and various procedures were developed for their synthesis.

Pyrazole and its derivatives represent one of the most active classes of heterocyclic compounds possessing a wide spectrum of biological activities. A literature survey reveals that a significant portion of research in heterocyclic chemistry has been devoted to pyrazoles containing different aryl groups as substituents [6]. For example, celecoxib (Figure 1) is a sulfonamide non-steroidal anti-inflammatory drug [7]. Considerable attention has been focused on pyrazoline derivatives, due to their interesting biological activities. They have been found to possess anti-oxidant, anti-cancer, anti-HIV, anti-malarial, anti-fungal, anti-bacterial, anti-amoebic, and anti-mycobacterial activities [8-9,11,12].

Ultrasound has increasingly been used in organic synthesis in the last three decades. A large number of organic reactions can be carried out in higher yields, shorter reaction time or milder conditions under ultrasound irradiation. The main aim of the present study is to synthesize, characterize and screen some 1,3,5-disubstituted pyrazolines carrying fluorine containing phenyl moiety. The structures of the various synthesized compounds were assigned on the basis of elemental analysis, IR and 1H NMR spectral data. These compounds were also screened for their antifungal activity

2. EXPERIMENTAL

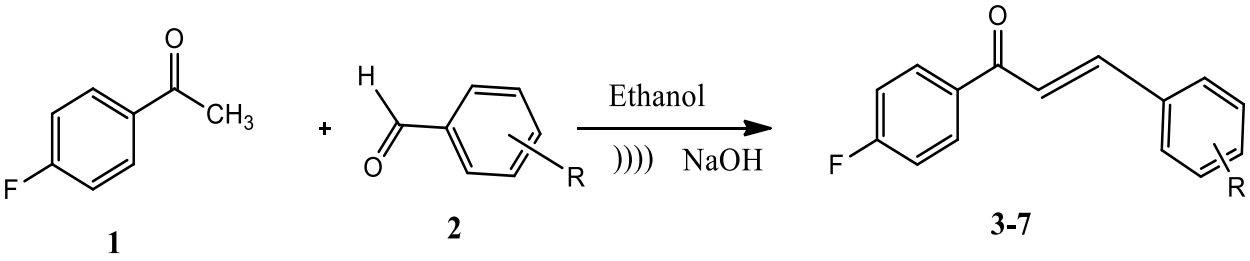
The melting points were taken in open capillary tube and are uncorrected. Infrared spectra (KBr, 4000-400 cm^{-1}) were recorded on AVATAR-300 Fourier transform spectrophotometer ^{1}H-NMR spectra were recorded on 400 MHz-BRUCKER using $CDCl_3$ as solvent. The chemical shifts are reported as parts per million downfield from tetra methyl silane (Me_4Si). The spectral data are presented in Table 1.

General procedure for preparation of (E)-1-(4-fluorophenyl)-3 substitutedphenylprop-2-en-1-one (3-7)

4-Fluoroacetophenone (2.5 mmol), Substituted benzaldehydes (2.5 mmol) 95% Ethanol (20 ml) and 2N NaOH (3 ml) were taken into a 100 ml conical flask. The mixture was irradiated in ultrasonic generator at room temperature for 3 min. The product was filtered with suction on a Buchner funnel, washed with cold water until the washings were neutral to litmus and then with ice cold ethanol. The crude product was recrystallized from ethanol.

Synthesis of methyl 3,4,5-trimethoxybenzoate

3,4,5-trimethoxy Benzoic acid (0.01 mol) in 20 ml of methanol and 0.5 ml conc. Sulphuric acid were taken into a 100 ml conical flask. The mixture was irradiated in ultrasonic generator at room temperature for 10 min. The product was filtered with suction on a Buchner funnel, washed with cold water. The product was isolated and treated with standard sodium bicarbonate solution to give desired compounds. m.p: 81-83 °C



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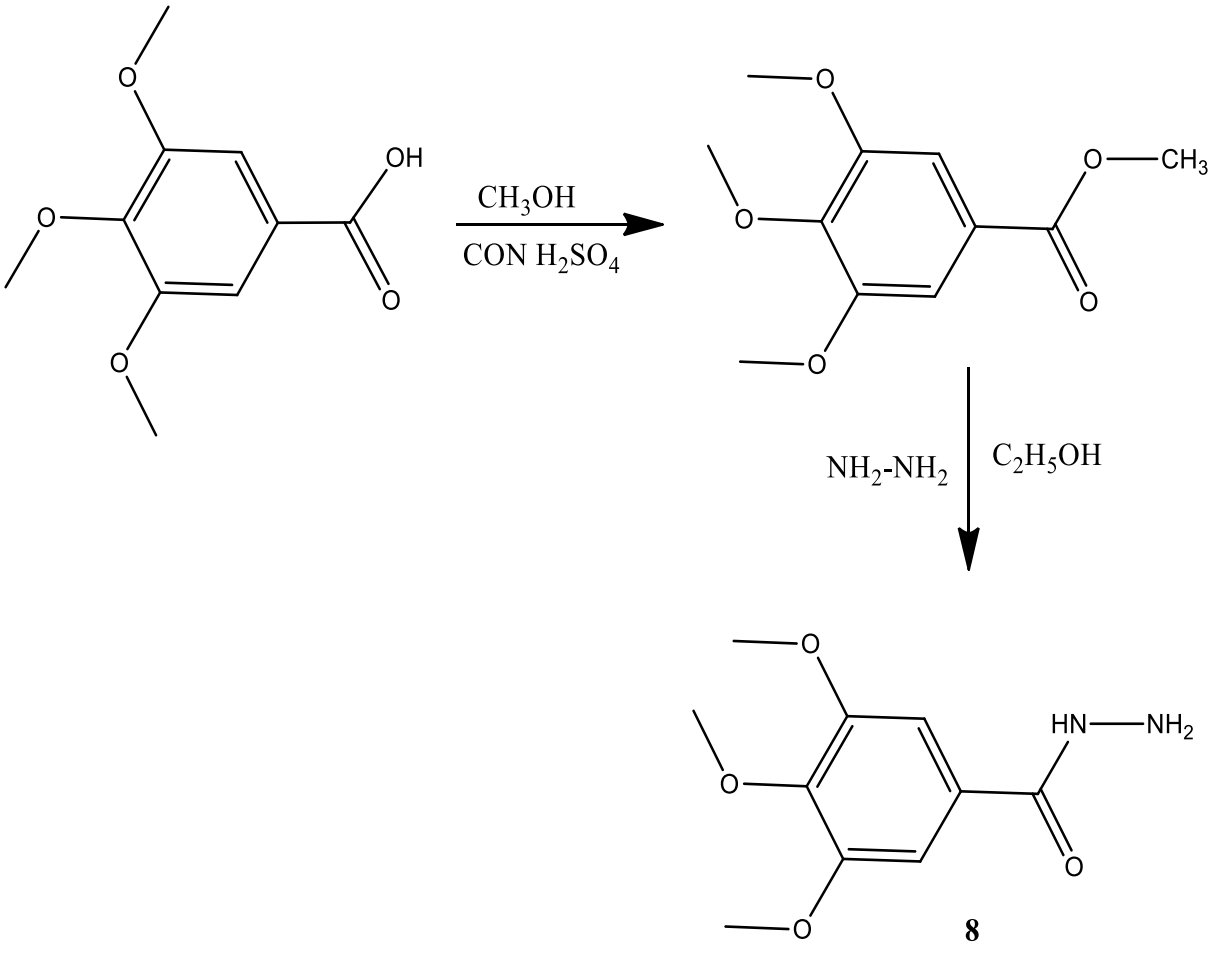
Synthesis of 3,4,5-trimethoxybenzohydrazide (8)

A mixture methyl 3,4,5-trimethoxybenzoate (0.01 mol) and hydrazine hydrate (0.5 g, 0.01 mole) were taken into a 100 ml conical flask. The mixture was irradiated in ultrasonic generator at room temperature for 20 min. The product was filtered with suction on a Buchner funnel, washed with cold water. The product was isolated and crystallized from ethanol. m.p = 156-160 °C; Mol. Formula: $C_8H_{10}N_2O_2$; Mol. wt: 166; IR (cm^{-1}): 3170 (NH str.), 3043 (Aromatic C-H str.), 1641(C=O str.), 2926 (Ali C-H str.),

Table 1. Physical Properties of
(E)-1-(4-fluorophenyl)-3 substitutedphenylprop-2-en-1-one (3-7)

Compound	X	Molecular formula	Molecular weight	Melting point	IR DATA
1	H	$C_{15}H_{11}FO$	226	80-82	C=C: 1597, C=O:1627, C-F:1002
2	Cl	$C_{15}H_{10}ClFO$	260	121-122	C=C: 1604, C=O:1654, C-F:902
3	F	$C_{15}H_{10}F_2O$	244	111-113	C=C: 1602, C=O:1660, C-F:1002
4	CH_3	$C_{16}H_{13}FO$	240	132-134	C=C: 1593, C=O:1654, C-F:987
5	OCH_3	$C_{16}H_{13}FO_2$	256	142-144	C=C: 1591, C=O:1653, C-F:1018



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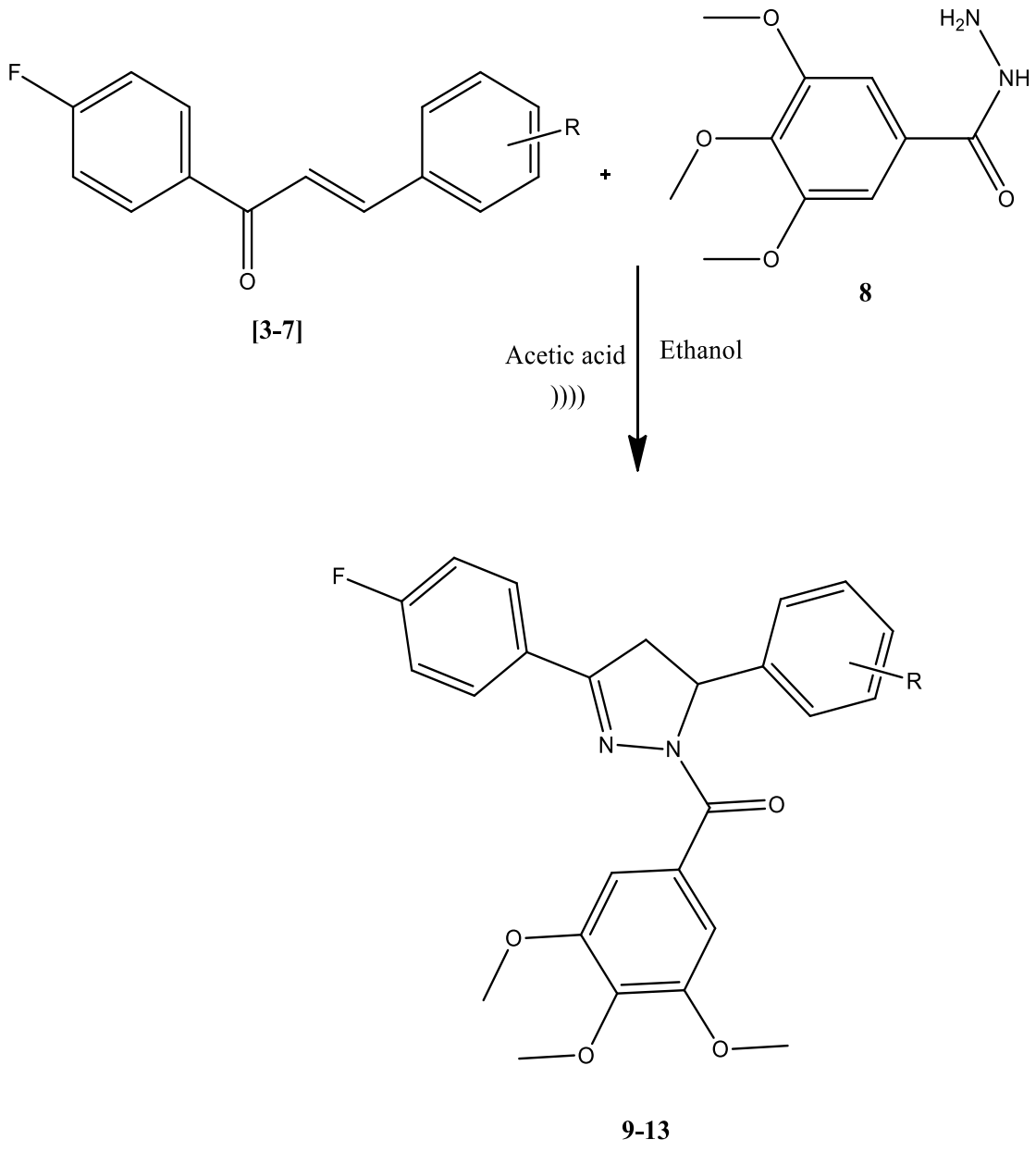
3,4,5-trimethoxybenzohydrazide

General procedure for preparation of (3-(4-fluorophenyl)-5-substitutedphenyl-4,5-dihydro-1H-pyrazol-1-yl)(3,4,5-trimethoxyphenyl)methanone (9-13)

Substituted chalcones (3-7) (2.5 mmol), 3,4,5-trimethoxybenzohydrazide (2.5 mmol) and glacial acetic acid (20 ml) were taken into a 100 ml conical flask. This reaction flask was suspended at the center of the ultrasonic cleaning-bath to get the maximum ultrasound energy and sonicated until crystals appeared or starting chalcone disappeared. The reaction-mixture was poured into crushed ice and left overnight. The precipitate was separated by filtration, washed well with water, dried and recrystallized from ethanol.



9-13

3. RESULT AND DISCUSSION

Synthesis of (Z)-N'-(3,3-dimethyl-2,6-diphenylpiperidin-4-ylidene)-methoxybenzohydrazide [8]

Mol. Formula: $C_{25}H_{23}FN_2O_4$; Mol. wt: 434.46; m.pt: 122-124 °C; IR (KBr) (cm^{-1}): 3062(Aro-C-H stretching), 2931 (Ali-C-H stretching), 1657 (C=O stretching), 1589 (C=N stretching); 1506 (C=C stretching) ^{1}H NMR(400 MHz, $CDCl_3$, δ,(ppm): 7.21.7.82 (m, Ar-H), 3.29 (dd, 1H, H_A), 3.46 (dd, 1H, H_B), 4.30 (dd, 1H, H_X), 3.54 (s, 9H, OCH_3),

Synthesis of (Z)-N'-(2,6-bis(4-fluorophenyl)-3,3-dimethylpiperidin-4-ylidene)-4-methoxy benzohydrazide [9]

Mol. Formula: $C_{25}H_{22}ClFN_2O_4$; Mol. wt: 468.9; m.pt: 251-53 °C; IR (KBr) (cm^{-1}): 3053(Aro-C-H stretching), 2931 (Ali-C-H stretching), 1687 (C=O stretching), 1591 (C=N stretching); 1508 (C=C stretching) ^{1}H NMR(400 MHz, $CDCl_3$, δ,(ppm): 7.26-8.01 (m, Ar-H), 3.01 (dd, 1H, H_A), 3.13 (dd, 1H, H_B), 4.58 (dd, 1H, H_X), 3.50 (s, 9H, OCH_3),

Synthesis of (Z)-N'-(2,6-bis(4-hydroxyphenyl)-3,3-dimethylpiperidin-4-ylidene)-4-methoxybenzohydrazide [10]

Mol. Formula: $C_{25}H_{22}F_2N_2O_4$; Mol. wt: 452.15; m.pt: 203-206 °C; IR (KBr) (cm^{-1}): 3062(Aro-C-H stretching), 2937 (Ali-C-H stretching), 1695 (C=O stretching), 1587 (C=N stretching); 1510 (C=C stretching) ^{1}H NMR(400 MHz, $CDCl_3$, δ,(ppm): 7.28-8.18 (m, Ar-H), 2.38 (dd, 1H, H_A), 2.54 (dd, 1H, H_B), 3.22 (dd, 1H, H_X), 3.68 (s, 9H, OCH_3),

Synthesis of (Z)-N'-(3,3-dimethyl-2,6-di-p-tolylpiperidin-4-ylidene)-4-methoxy benzohydrazide [11]

Mol. Formula: $C_{26}H_{25}FN_2O_4$; Mol. wt: 448.49; m.pt: 190-192 °C; IR (KBr) (cm^{-1}): 3091(Aro-C-H stretching), 2924 (Ali-C-H stretching), 1666 (C=O stretching), 1589 (C=N stretching) ; 1519 (C=C stretching) ^{1}H NMR(400 MHz, $CDCl_3$, δ,(ppm): 7.28-8.18 (m, Ar-H), 2.19 (dd, 1H, H_A), 3.37 (dd, 1H, H_B), 3.53 (dd, 1H, H_X), 3.68 (s, 9H, OCH_3), 2.61 (s, 3H, -CH_3),

Synthesis of (Z)-N'-(2,6-bis(4-methoxyphenyl)-3,3-dimethylpiperidin-4-ylidene)-4-methoxybenzohydrazide [12]

Mol. Formula: $C_{26}H_{25}FN_2O_5$; Mol. wt: 464.49; m.pt: 141-143 °C; **IR (KBr) (cm^{-1})**: 3032(Aro-C-H stretching), 2927 (Ali-C-H stretching), 1660 (C=O stretching), 1589 (C=N stretching); 1471 (C=C stretching) **^{1}H NMR(400 MHz, $CDCl_3$, δ,(ppm)**: 7.02-7.39 (m, Ar-H), 3.74 (dd, 1H, H_A), 4.02 (dd, 1H, H_B), 4.28 (dd, 1H, H_X), 3.99 (s, 12H, OCH_3),

4. ANTIFUNGAL ACTIVITY

Materials and methods

All those compounds screened earlier for antibacterial activity were also tested for their antifungal activity. The fungi employed for the screening were Aspergillusflavus, Aspergillusniger, Penicillimchryogenum, Trigodermaveride and Fusariumoxysporum.. Amphotericin -B was employed as standard to compare the results. The test organisms were sub-cultured using Potato-Dextrose-Agar (PDA) medium. The tubes containing sterilized medium were inoculated with test fungi and kept at room temperature for obtaining growth. After that, they were stored at 4 °C in a refrigerator.

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml, Analar grade) to give a concentration of 1000 µg/ml. Amphotericin -B solution was also prepared at a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained at 2 to 3 by using conc. HCl. All the compounds were tested at dose levels of 200 µg (0.2 ml) and DMSO used as a control. The solutions of each test compound, control and reference standards were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into

the PDA medium. Petri dishes were subsequently kept at room temperature for 48 hours. After that, the diameter of zone of inhibition in mm surrounding each of the cups was measured with the help of an antibiotic zone reader. The results are presented in Table 2.

A filter paper disc method was employed for the *in- vitro* study of antifungal effects against *Aspergillusflavus, Aspergillusniger, Penicillimchryogenum, Trigodermaveride and Fusariumoxysporum*. The results of this evaluation were compared with Amphotericin –B as reference standard. The antifungal activity of the pyrazoline derivatives are shown in Fig: 1, and the zone of inhibition values are given Table 2. The figure 1 Showed that pyrazoline derivatives of (9) to (13) posses significant activity almost equipotent with the standard Amphotericin –B. Thus the halogen substituted derivatives place a vital role in imparting enhanced antifungal activity to the compounds. The screening results indicate that compounds (9) and (12) were found to be active against *Aspergillus flavus*. Compounds (11) was found to moderately active be active against *Aspergillus flavus*. Compounds (10) and (12) were found to be active against *Aspergillus niger* .

Table 2. Antifungal activity of 1,3,5-trisubstituted pyrazoline derivatives (9-13)
By disc diffusion method

S. No.	Bacteria	Standard Antibiotic Disk*	Zone of inhibition mm in diameter					
			1	2	3	4	5	Control (DMSO)
1	*Aspergillus flavus*	20	9	6	8	9	6	-
2	*Aspergillus niger*	21	0	11	6	12	0	-
3	*Fusarium oxysporum*	18	6	7	9	8	0	-
4	*Penicillim chryogenum*	15	8	7	6	9	7	-
5	*Trigoderma veride*	15	7	8	0	6	7	-

Compound (12) was found to active against *Penicillim chryogenum.* Compounds (11) was found to be moderately active against *Penicillim chryogenum*, Compound (10) was found to be active against *Trigoderma veride*. Compounds (9) and (13) were found to be moderately active against *Trigoderma veride.* Compound (11) was found to be active against *Fusarium oxysporum*. Compounds (10) and (12)were found to be moderately active against *Fusarium oxysporum.*

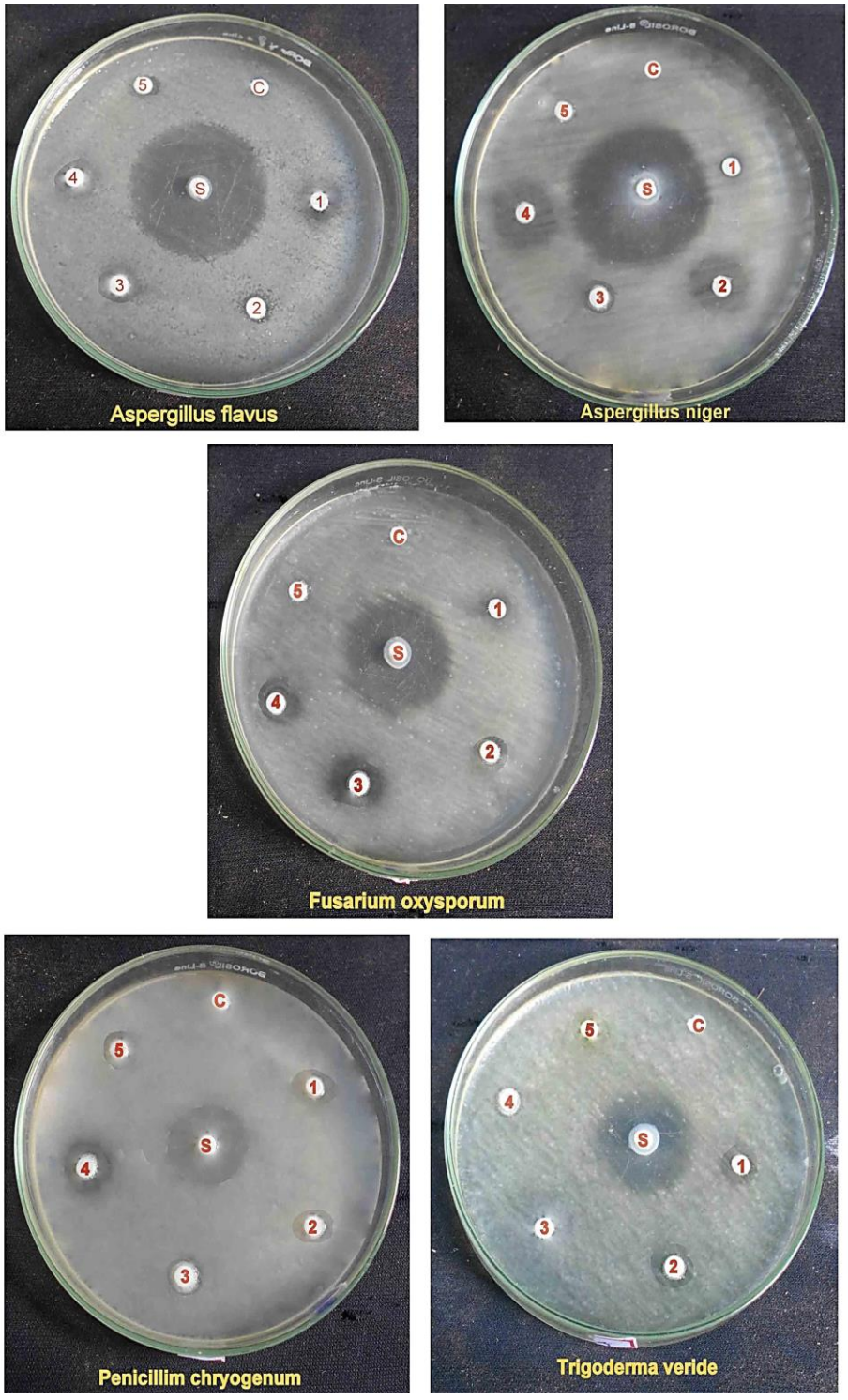


Fig. 1. Antifungal activity of 1,3,5-trisubstituted pyrazoline derivatives (9-13) By disc diffusion method

5. CONCLUSION

In conclusion, the ultrasonic irradiation method was proved to be a better method as it produced much higher yield than the conventional method and synthesis provides an excellent approach for the safe, rapid, economical, environment friendly, non-hazardous, and easier work-up procedure. All the synthesized compounds were characterized by IR, NMR spectra studies. Their purity was established by TLC. All the synthesized compounds of different classes were evaluated for antifungal Activity studies. Halogen substituted derivatives **10** and **11** exhibited good activity compare with the standard drug.

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