



Synthesis, Characterization of Some New 1,3,5-Trisubstituted Pyrazole Derivatives for Their Antifungal Potential

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The aim of the study was to develop, synthesis, and characterise a novel 1,3,5-trisubstituted-2-pyrazolines derivative, as well as to evaluate its antifungal activity. The reaction of chalcone derivatives with succinic hydrazide in the presence of pyridine yielded the 1,3,5-tri-substituted-2-pyrazolines derivatives. Total 20 compounds has been synthesized and characterized by the IR, 1H NMR and mass spectral analysis. Antifungal activity of the compounds carried out on four fungal strains i.e. *Saccharomyces cerevisiae*, *A. Niger*, *C. Albicans* and *R. Oryzae* in two different concentration i.e. 50 and 100 µg/ml by Agar-diffusion method using Cup-plate method. The usual antifungal medicine was ketoconazole. All of the synthesized 1,3,5-trisubstituted pyrazole compounds (ME1-ME8, CL1-CL8, BR1-BR4) showed medium to best action against examined organisms, according to antifungal activity data. The antifungal activity of compounds against fungal strains (*Saccharomyces cerevisiae*, *A. Niger*, *Candida albicans*, and *R. Oryzae*) revealed the following order of action: CL-4 > BR-4 > CL-3 > CL-2 > ME-3 > ME-2 > CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8 > ME-8 > CL-1 > ME-1 > CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8 > ME-8 > CL-1 > ME-1. Electronegative groups (Br, Cl, F, and NO₂) must be present at the third and fifth positions of the 1,3,5-pyrazoline ring for significant antifungal action. The presence of an electronegative group at the third and fifth positions may be required for the best

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action against bacterial and fungal strains, however the addition of F, NO₂ has demonstrated moderate activity, while the substitution of methyl and methoxy groups may reduce the activity. The synthesized compounds in the BR-1 through BR-4 class are the most active.

Keywords: Antifungal; Ketoconazole; agar diffusion; pyrazole; cup plate method.

1. INTRODUCTION

In the contemporary period, sickness from fungal and bacterial origins has increased dramatically. There is an increased need for novel medications or chemically modified moieties that are effective against bacterial and fungal illness. We discovered the diazoles, which are claimed to be employed as antifungal medicines, while searching for moieties that must be efficient against fungal infection [1]. Anti-fungals are used to treat fungal diseases including athlete's foot, ringworm, and candidiasis (thrush), as well as dangerous systemic infections such cryptococcal meningitis. Antifungal kills the fungal organism without harming the host by taking advantage of distinctions between mammalian and fungal cells. As a result, novel moieties are being sought to aid in the treatment of fungal infections [2]. Pyrazole derivatives have the interest of chemists due to their vast variety of biological characteristics and many practical uses. Pyrazole has been shown to have a wide range of biological effects, including antifungal, anticancer, anti-inflammatory, antifungal, anti-proliferative, anticonvulsant, antioxidant, and antitubercular properties. The pyrazole ring is a useful synthetic target in the pharmaceutical industry because of its wide variety of biological activities [3]. Pyrazole-containing products are commercially available. Celecoxib is an NSAID used to treat rheumatoid arthritis, osteoarthritis, acute pain, and menstruation; Phenzone (phenazon or antipyrine) is an analgesic and antipyretic; Lonazolac is an NSAID; Betazole is an H₂ receptor agonist. It is used to assess stomach secretory function in clinical settings. Fipronil is a broad-spectrum pesticide that affects the central nervous system of insects by inhibiting chloride ions from passing through the gamma-Aminobutyric acid (GABA) receptor and glutamate-gated chloride channels, which are both central nervous system components [4]. Chalcone is made through claisen-schmidt condensation of an aromatic aldehyde and a ketone, which is catalyzed by a base or an acid, and then dehydration. Antifungal action is mediated by the presence of a,-unsaturated functional group, which can be changed depending on the kind of substituent on the aromatic rings [5]. As they undergo a range of

chemical processes, they also serve as a backbone for the production of numerous heterocyclic compounds. As a result, chalcone plays a crucial role in the production of therapeutic substances [6]. Antifungal agents, antioxidants, anti-inflammatory activity, cytotoxic activity, hypoglycaemic activity, anti-hepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors, and antitumor activities are among the pharmacological activities of many chalcone derivatives of natural or synthetic origin, according to a review of the literature [7].

The combined synthesis reaction of chalcone with pyrazole moieties may be useful in synthesizing a novel pyrazole derivative, which will aid in the hunt for new antifungal compounds. The aim of the study was to see how effective novel 1,3,5-trisubstituted-2-pyrazolines derivatives in fighting fungus.

2. MATERIALS AND METHODS

Hi-media, New Delhi, provided the chemicals p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone. CDH (Chemical Drug House) in New Delhi, India, provided benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methylbenzaldehyde, and 4-methoxybenzaldehyde. Sigma Aldrich, New Delhi, provided the succinic acid. Chemicals of synthetic grade were utilized in the experiments. In open glass capillaries, the melting points of the produced compounds were determined. The Bruker-alpha IR Spectrometer was used to record IR spectra. Elemental analysis was carried out, and the results were determined to be within 0.4 percent of the theoretical values. ¹H NMR spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5mm multi-nuclear inverse probe head, low and high-temperature facility. Mass Spectra were recorded using Mass Spectrometers Jeol SX-102 (FAB) by ESI.

2.1 Chemistry

Present synthesis comprises

Synthesis of 1,3,5-trisubstituted pyrazole derivatives involves the following steps.

Scheme-I: Synthesis of chalcones by claisen-schmidt condensation

Scheme-II: Synthesis of Succinic hydrazide from correspondingester

Scheme-III: Reaction of Succinic hydrazide with chalcone to form 1,3,5-trisubstituted pyrazolederivatives

Scheme I: Synthesis of chalcones by claisen-schmidt condensation

An equimolar amount of substituted acetophenone (0.05 M) was combined with an equimolar amount of benzaldehyde and substituted benzaldehyde. In ethanol, the mixture was dissolved. The mixture was stirred for 5 minutes before adding a 50 percent aqueous potassium hydroxide solution slowly and stirring for 24 hours at room temperature. The thin layer chromatography (TLC) was used to monitor the reaction's completion. The reaction was then finished and poured over crushed ice, yielding a solid product; however, if the solid product was not produced, it was acidified with dilute hydrochloric acid [8]. The obtained solid was separated by filtration, dried and purified by column chromatography using solvent system (hexane: ethyl acetate). The reaction was shown in synthesis Scheme I.

Scheme II: Synthesis of succinic hydrazide

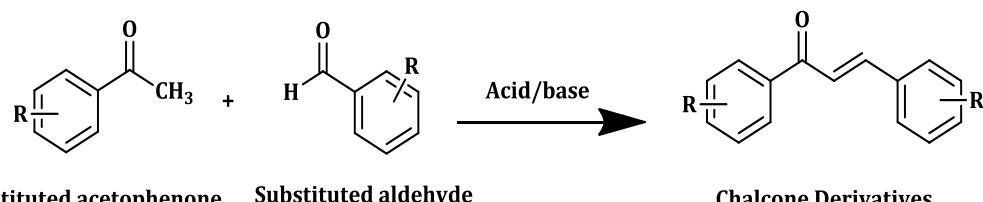
Succinic acid (0.05M) may be readily transformed to succinic hydrazide by reacting it

with hydrazine hydrate (0.05M) in alcohol, cooling the reaction mixture to room temperature, and then recrystallizing the succinic hydrazide as a solid. The IR spectra show the peak at 3500.66 (-NH str.); 3313.58 (NH₂ str.); 1658.32 (C=O); 1430-3046.55 (-NH str.); 1430-3046.55 (-NH str.); 1430-3046.55 (-NH str.); 1430-3046.55 (-CH-CH). TLC was used to monitor the reaction, which used Hexane: ethyl acetate as the mobile phase. The obtained compounds were analysed using IR and ¹H NMR and found to have a structure that was compatible with what was predicted. Scheme-II depicted the synthesis.

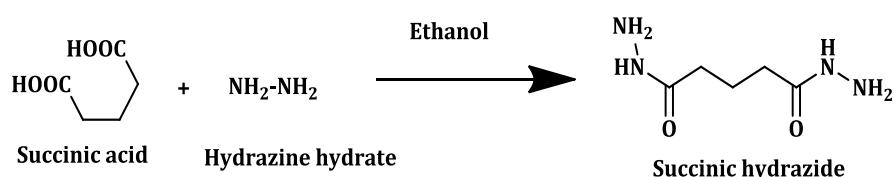
Scheme III: Synthesis of 1,3,5-tri substituted pyrazole

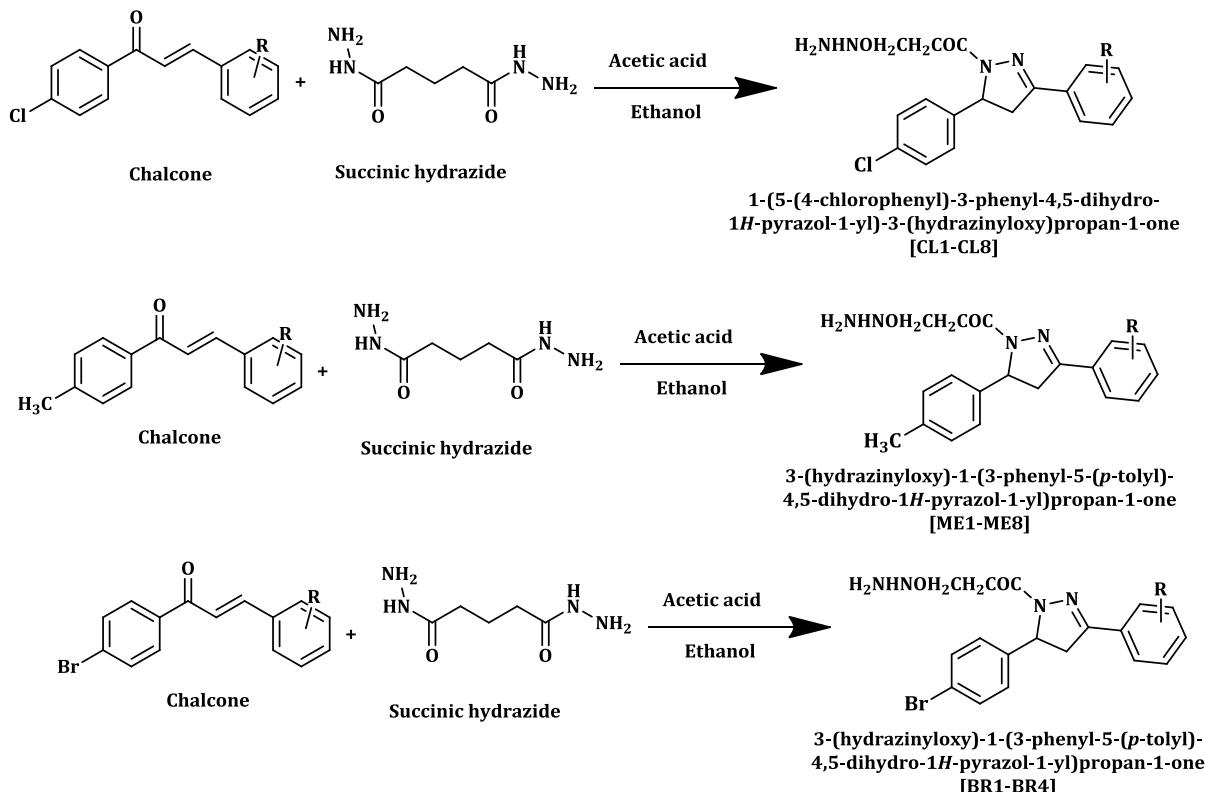
The synthesized chalcone derivatives were combined in absolute alcohol with equimolar amounts of succinic hydrazide (0.005 M) and a little amount of pyridine (0.01M). The reaction mixture was refluxed for 2-6 hours at 65°C. TLC was used to monitor the reaction, which used ethyl acetate:hexane as the mobile phase. The solvent was entirely evaporated before being placed into ice cold water and constantly stirred to turn the liquid into a solid product, which yielded the synthesized product [9]. This substance was filtered and dried under vacuum. The synthesized compound purified by the column chromatography and were obtained as pale yellow solid colour powder. The synthesis was shown in Scheme-III.

Scheme-I



Scheme-II



Scheme-III

2.2 Evaluation of Antifungal Activity

In order to study and evaluate the antifungal activity, reliable and reproducible methods must be used to evaluate them. Agar Diffusion method was used for evaluation of the antifungal activity using Cup-plate method [10-13].

2.3 Antifungal Screening of the Synthesized Compounds

The antifungal activity of all 20 produced compounds was tested. *Saccharomyces cerevisiae*, *Aspergillus NIGER*, *Rhizopus oryzae*, and *Candida albicans* were obtained from IMTECH Chandigarh for the screening. As a standard, ketoconazole was used. Potato-DextroseAgar (PDA) medium was used to subculture the test organisms [14-17]. To acquire growth, the tubes containing sterilized media were injected with test fungus and stored at room temperature. They were then refrigerated at 4 degrees Celsius. Cup plate technique was used to test the activity of the derivatives at various concentration levels. The typical medicine was ketoconazole. Each test chemical (5 mg) was diluted in 5 mL Analar grade dimethyl sulfoxide to provide a 1000 g/mL concentration. Each test

compound's solution, control, and reference standards (0.05 ml and 0.1 ml) were put individually in the cups, and the plates were left undisturbed in the refrigerator for at least 2 hours to allow appropriate diffusion of the solution into the PDA medium. After that, the petri dishes were stored at room temperature for 48 hours. After that, using an antibiotic zone reader, the diameter of the zone of inhibition in mm surrounding each of the cups was measured.

3. RESULTS AND DISCUSSION

Scheme I: The synthesized compounds was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, ¹H NMR and were found consistent with an expected structure. The IR spectra denotes the peak at 1650-1658 (C=O); 1500-1580 (C=C Quadrant of Ar), 761 (mono substituted benzene); 1105 (C-F), 825 (C-Cl), 1015 (C-Br), and 1160 (OCH₃). These compound further confirmed by proton NMR revealed the characteristic ethylene protons of the chalcone system in between δ 7.60 (C=O-CH), 6.68-7.90 (Ar-H) and 8.05 (=CH-Ar) confirm the compound. The reaction was monitored by

the TLC using Hexane: ethyl acetate as mobile phase.

Scheme III: The synthesized compounds was characterized by the Infra-red spectroscopy and proton NMR spectroscopy and was found reliable with probable structure. Obtained compounds were characterized by IR, 1HNMR and were found consistent with an expected structure. The IR spectra demotes the peak at 3205.66 (C-H str., aromatic) 1510.25 (C=N), 3042.55 (C-H), 1660.32 (C=O), 1486.20 (C=N), 3502.21 (-NH str.) and 3315.50 (-NH₂ str.), 852.22 (C-Cl), 1025.27 (C-Br), 1118.62 (C-F), 1072.46 (C-OCH₃), 1569 (N=O str.) and 1365 (N-O str.). These compound further confirmed by proton NMR revealed the characteristic protons of the system δ 1.26, 1.28 (4H methylene of pyrazoline), δ 4.81 (4H methylene side chain of pyrazoline), δ 3.60 (1H, dd, pyrazole ring); δ 5.38 (methyl group at phenyl ring), δ 1.50-158 (NH₂) and 8.33 (N-H) confirm the compound. The reaction was monitored by the TLC using Hexane: ethyl acetate as mobile phase.

Compound ME-1: 3-(hydrazinyloxy)-1-(3-phenyl-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one

Molecular formula: C₁₉H₂₂N₄O₂; Molecular weight: 338.40; TLC (Rf value): 0.45; Element (Found/Calc. %): Nitrogen (16.52/16.56); Oxygen (9.45/9.46); IR (cm⁻¹): 3205.66 (C-H str.), 1510.25(C=N str.), 1172.05 C₆H₅, 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH₂ str.); 1HNMR (ppm): δ 1.32 (4H methylene of pyrazoline), δ 4.81 (4H methylene side chain of pyrazoline), δ 3.69 (1H, dd, pyrazole ring); δ 2.15 (methyl group at phenyl ring), δ 1.55 (NH₂), 8.30 (N-H), δ 7.10–7.20 (m, 2H, Ar-H), δ 7.52–7.67 (m, 3H, Ar-H).FAB Mass (m/z): 338.17 (Quassi-molecular ion peak (M+H)+).

Compound ME-2: 1-(3-(4-fluorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₂₂H₂₀CIN₃O₄S; Molecular weight 356.39; TLC (Rf value): 0.38; Element (Found/Calc. %): Nitrogen (9.12/9.18); Oxygen (13.95/13.98); IR (cm⁻¹): 3202.46 C-H str., 1510.15(C=N str.), 3038.47 (C-H str.), 1658.34 (C=O str.), 1482.25 (C=N str.), 3515.41 (-NH str.), 3310.20 (-NH₂ str.), 1118.62 (C-F); 1HNMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ

3.65 (1H, dd, pyrazole ring); δ 5.38 (methyl group at phenyl ring), δ 1.54 (NH₂), 8.32 (N-H), δ 7.10–7.20 (m, 2H, Ar-H), δ 7.36–7.81 (m, 3H, Ar-H).FAB Mass (m/z): 356.16 (Quassi-molecular ion peak (M+H)+)

Compound ME-3: 1-(3-(4-chlorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₉H₂₁ClN₄O₂; Molecular weight: 372.85; TLC (Rf value): 0.40; Element (Found/Calc. %): Nitrogen (15.00/15.02); Oxygen (8.56/8.58); IR (cm⁻¹): 3202.46 (C-H str.), 1520.30(C=N str.), 3040.55 (C-H str.), 1658.32 (C=O str.), 1482.48(C=N str.), 3506.16 (-NH str.), 3312.42 (-NH₂ str.), 850.22(C-Cl); 1HNMR: δ 1.27 (4H methylene of pyrazoline), δ 4.84 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.52 (NH₂), 8.32 (N-H), δ 7.12–7.20 (m, 2H, Ar-H), δ 7.52–7.95 (m, 3H, Ar-H).FAB Mass (m/z): 372.14 (Quassi-molecular ion peak (M+H)+)

Compound ME-4: 1-(3-(4-bromophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₉H₂₁BrN₄O₂; Molecular weight: 417.30; TLC (Rf value): 0.54; Element (Found/Calc. %): Nitrogen (13.40/13.43); Oxygen (7.65/7.67); IR (cm⁻¹): 3206.32 (C-H str.), 1509.26(C=N str.), 3040.52 (C-H str.), 1658.30 (C=O str.), 1482.30 (C=N str.), 3509.16 (-NH str.), 3312.40 (-NH₂ str.), 1020.27 (C-Br); 1HNMR (ppm): δ 1.22 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.58 (NH₂), 8.29 (N-H), δ 7.15–7.20 (m, 2H, Ar-H), δ 7.58–7.72 (m, 2H, Ar-H).FAB Mass (m/z): 416.08 (Quassi-molecular ion peak (M+H)+)

Compound ME-5: 3-(hydrazinyloxy)-1-(3-(4-nitrophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one

Molecular formula: C₁₉H₂₁N₅O₄; Molecular weight: 383.40; TLC (Rf value): 0.30; Element (Found/Calc. %): Nitrogen (18.25/18.27); Oxygen (16.65/16.69); IR (cm⁻¹): 3202.66 (C-H str.), 1512.20(C=N str.), 3038.35 (C-H str.), 1658.32 (C=O str.), 1476.20 (C=N str.), 3509.21 (-NH str.), 3312.50 (-NH₂ str.), 1562.25(N=O str.), 1362.42 (N-O str.); 1HNMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H

methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.52 (NH₂), 8.30 (N-H), δ 7.10–7.20 (m, 2H, Ar-H), δ 8.09–8.33 (m, 2H, Ar-H).FAB Mass (m/z): 333.40 (Quassi-molecular ion peak (M+H)+)

Compound ME-6: 1-(3,5-di-p-tolyl-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₂₀H₂₄N₄O₂; Molecular weight: 352.43; TLC (Rf value): 0.64; Element (Found/Calc.)%: Nitrogen (9.58/9.60); Sulphur (7.32/7.33); Oxygen (14.60/14.63); IR (cm⁻¹): 3206.66 (C-H str.), 1512.23(C=N str.), 3040.34 (C-H str.), 1658.32 (C=O str.), 1482.20 (C=N str.), 3506.21 (-NH str.), 3312.50 (-NH₂ str.); 1HNMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 2.12 (methyl group at phenyl ring), δ 1.53 (NH₂), 8.29 (N-H), δ 7.10–7.20 (m, 2H, Ar-H), δ 7.25–7.71 (m, 2H, Ar-H).FAB Mass (m/z): 352.19 (Quassi-molecular ion peak (M+H)+)

Compound ME-7: 3-(hydrazinyloxy)-1-(3-(4-methoxyphenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one

Molecular formula: C₂₀H₂₄N₄O₃; Molecular weight: 368.43; TLC (Rf value): 0.23; Element (Found/Calc.)%: Nitrogen (15.19/15.21); Oxygen (13.01/13.03); IR (cm⁻¹): 3208.66 (C-H str.), 1514.25(C=N str.), 3040.55 (C-H str.), 1662.32 (C=O str.), 1485.15 (C=N str.), 3506.18 (-NH str.), 3312.35 (-NH₂ str.), 1074.26 (-OCH₃); 1HNMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.66 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.56 (NH₂), 8.53 (N-H), δ 7.10–7.20 (m, 2H, Ar-H), δ 7.30–7.80 (m, 2H, Ar-H), δ 3.81 (-OCH₃).FAB Mass (m/z): 368.18 (Quassi-molecular ion peak (M+H)+)

Compound ME-8: 1-(3-(4-(dimethylamino)phenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₂₁H₂₇N₅O₂; Molecular weight: 381.47; TLC (Rf value): 0.42; Element (Found/Calc.)%: Nitrogen (18.35/18.36); Oxygen (8.37/8.39);IR (cm⁻¹): 3204.42 (C-H str.), 1511.38(C=N str.), 3040.22 (C-H str.), 1658.16 (C=O str.), 1455.18 (C=N str.), 3510.15 (-NH str.),

3312.42 (-NH₂ str.); 1HNMR (ppm): δ 1.28 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.69 (1H, dd, pyrazole ring); δ 2.18 (methyl group at phenyl ring), δ 1.50–158 (NH₂), 8.33 (N-H), δ 7.15–7.20 (m, 2H, Ar-H), δ 6.68–7.50 (m, 2H, Ar-H), δ 2.58 (N(CH₃)₂). FAB Mass (m/z): 381.47 (Quassi-molecular ion peak (M+H)+)

Compound CL-1: 1-(5-(4-chlorophenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₈H₁₉ClN₄O₂; Molecular weight: 358.82; TLC (Rf value): 0.38; Element (Found/Calc.) %: Nitrogen (15.60/15.61); Oxygen (8.90/8.92); IR (cm⁻¹): 3206.66 (C-H str.), 1172.05-C₆H₅, 1512.25(C=N str.), 3042.55 (C-H str.), 1665.32 (C=O str.), 1482.20 (C=N str.), 3502.21(-NH str.), 3312.50(-NH₂ str.), 852.22(C-Cl); 1HNMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.68 (1H, dd, pyrazole ring); δ 1.56 (NH₂), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.52–7.67 (m, 2H, Ar-H).FAB Mass (m/z): 344.12 (Quassi-molecular ion peak (M+H)+).

Compound CL-2: 1-(5-(4-chlorophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₈H₁₈CIFN₄O₂; Molecular weight: 376.81; TLC (Rf value): 0.42; Element (Found/Calc.)%: Nitrogen (14.85/14.87); Oxygen (8.48/8.49); IR (cm⁻¹): 3215.66 (C-H str.) 1506.25(C=N str.), 3032.55 (C-H str.), 1640.32 (C=O str.), 1466.20 (C=N str.), 3509.21 (-NH str.) 3312.50 (-NH₂ str.).850.22 (C-Cl), 1118.62(C-F); 1HNMR (ppm): δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring), δ 1.56 (NH₂), δ 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.36–7.81 (m, 2H, Ar-H).FAB Mass (m/z): 376.11 (Quassi-molecular ion peak (M+H)+).

Compound CL-3: 1-(3,5-bis(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinylloxy)propan-1-one

Molecular formula: C₁₈H₁₈Cl₂N₄O₂; Molecular weight: 393.27; TLC (Rf value): 0.40; Element (Found/Calc.)%: Nitrogen (14.24/14.25); Oxygen (8.12/8.14); IR (cm⁻¹): 3208.66 (C-H str.), 1512.35(C=N str.), 3052.45 (C-H str.), 1640.32 (C=O str.), 1456.20 (C=N str.), 3515.41 (-NH str.),

3310.20 (-NH₂ str.), 852.22(C-Cl); 1HNMR (ppm):δ 1.28 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring), δ 1.56 (NH₂), 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.52–7.98 (m, 2H, Ar-H).FAB Mass (m/z): 392.08 (Quasi-molecular ion peak (M+H)+).

Compound CL-4: 1-(3-(4-bromophenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₈H₁₈BrClN₄O₂; Molecular weight: 437.72; TLC (Rf value): 0.45; Element (Found/Calc.)%: Nitrogen (12.78/12.80); Oxygen (7.30/7.31); IR (cm⁻¹): 3212.56 (C-H str.), 1514.15(C=N str.), 3040.45 (C-H str.), 1658.22 (C=O str.), 1479.10 (C=N str.), 3509.16 (-NH str.), 3314.40 (-NH₂ str.), 850.12 (C-Cl), 1020.37 (C-Br); 1HNMR(ppm):δ 1.25 (4H methylene of pyrazoline), δ 4.78 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.56 (NH₂), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.58–7.72 (m, 2H, Ar-H).FAB Mass (m/z): 438.03 (Quasi-molecular ion peak (M+H)+)

Compound CL-5: 1-(5-(4-chlorophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₈H₁₈CIN₅O₄; Molecular weight: 403.82; TLC (Rf value): 0.36; Element (Found/Calc.)%: Nitrogen (17.32/17.34); Oxygen (15.80/15.85); IR (cm⁻¹): 3205.66 (C-H str.), 1512.25(C=N str.), 3040.55 (C-H str.), 1660.32 (C=O str.), 1482.20 (C=N str.), 3509.21 (-NH str.), 3318.50 (-NH₂ str.), 850.22(C-Cl), 1564.62 (N=O str.), 1362.52 (N-O str.); 1HNMR (ppm):δ 1.24 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 1.58 (NH₂), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 8.10–8.33 (m, 2H, Ar-H).FAB Mass (m/z): 403.10 (Quasi-molecular ion peak (M+H)+)

Compound CL-6: 1-(5-(4-chlorophenyl)-3-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₉H₂₁CIN₄O₂; Molecular weight: 372.85; TLC (Rf value): 0.32; Element (Found/Calc.)%: Nitrogen (15.02/15.03); Oxygen (8.56/8.58); IR (cm⁻¹): 3212.42 (C-H str.) 1512.42(C=N str.), 3040.52 (C-H str.), 1658.66 (C=O str.), 1474.40 (C=N str.), 3509.25 (-NH str.) 3312.40 (-NH₂ str.).850.22(C-Cl); 1HNMR (ppm):δ 1.28 (4H methylene of pyrazoline), δ

4.80 (4H methylene side chain of pyrazoline), δ 3.58 (1H, dd, pyrazole ring); δ 2.15 (methyl group at phenyl ring), δ 1.56 (NH₂), 8.30 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.28–7.68 (m, 2H, Ar-H).FAB Mass (m/z): 372.14 (Quassi-molecular ion peak (M+H)+)

Code No: CL-7: 1-(5-(4-chlorophenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₉H₂₁CIN₄O₃; Molecular weight: 388.85; TLC (Rf value): 0.30; Element (Found/Calc.)%: Nitrogen (14.40/14.41); Oxygen (12.32/12.34); IR (cm⁻¹): 3212.66 (C-H str.), 1512.25(C=N str.), 3040.55 (C-H str.), 1664.32 (C=O str.), 1485.20 (C=N str.), 3509.21 (-NH str.), 3314.50 (-NH₂ str.), 850.22 (C-Cl str.), 1072.46(-OCH₃); 1HNMR (ppm):δ 1.28 (4H methylene of pyrazoline), δ 4.83 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.56 (NH₂), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 7.30–7.70 (m, 2H, Ar-H), δ 3.81 (-OCH₃).FAB Mass (m/z): 388.13 (Quassi-molecular ion peak (M+H)+)

Compound CL-8: 1-(5-(4-chlorophenyl)-3-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₂₀H₂₄CIN₅O₂; Molecular weight: 401.89; TLC (Rf value) 0.48; Element (Found/Calc.)%: Nitrogen (17.42/17.43); Oxygen (7.95/7.96); IR (cm⁻¹): 3209.66 (C-H str.) 1512.25(C=N str.), 3040.55 (C-H str.), 1662.32 (C=O str.), 1481.20 (C=N str.), 3504.21 (-NH str.), 3315.50 (-NH₂ str.), 850.22(C-Cl); 1HNMR (ppm):δ 1.26 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ 3.65 (1H, dd, pyrazole ring); δ 1.54 (NH₂), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar-H), δ 6.65–7.50 (m, 2H, Ar-H), 2.58 (N(CH₃)₂).FAB Mass (m/z): 401.16 (Quassi-molecular ion peak (M+H)+).

Compound BR-1: 1-(5-(4-bromophenyl)-3-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyloxy)propan-1-one

Molecular formula: C₁₈H₁₈BrFN₄O₂; Molecular weight: 421.26; TLC (Rf value): 0.44; Element (Found/Calc.)%: Nitrogen (13.28/13.30); Oxygen (7.58/7.60); IR (cm⁻¹): 3205.66(C-H str.), 1510.25(C=N str.), 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH₂ str.), 1025.27 (C-Br), 1118.62 (C-F); 1HNMR (ppm):δ 1.26 (4H methylene of pyrazoline), δ

4.80 (4H methylene side chain of pyrazoline), δ 3.68 (1H, dd, pyrazole ring); δ 1.58 (NH₂), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar-H), δ 7.52–7.81 (m, 2H, Ar-H).FAB Mass (m/z): 420.06 (Quasi-molecular ion peak (M+H)+).

Compound BR-2: 1-(5-(4-bromophenyl)-3-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyl)propan-1-one

Molecular formula: C₁₈H₁₈BrClN₄O₂; Molecular weight: 437.72; TLC (Rf value): 0.54; Element (Found/Calc.)%: Nitrogen (12.78/12.80); Oxygen (7.28/7.31); IR (cm⁻¹):3208.26 (C-H str.), 1512.45(C=N str.), 3040.35 (C-H str.), 1658.22 (C=O str.),1478.44 (C=N str.),3509.25 (-NH str.), 3310.35 (-NH₂ str.), 1028.22 (C-Br), 850.25(C-Cl);¹HNMR (ppm):δ 1.26 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.54 (NH₂), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar-H), δ 7.52–7.75 (m, 2H, Ar-H).FAB Mass (m/z): 438.03 (Quasi-molecular ion peak (M+H)+).

Compound BR-3: 1-(3,5-bis(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyl)propan-1-one

Molecular formula: C₁₈H₁₈Br₂N₄O₂; Molecular weight: 482.17; TLC (Rf value): 0.55; Element (Found/Calc.)%: Nitrogen (11.60/11.62); Oxygen (6.62/6.64); IR (cm⁻¹): 3215.45 (C-H str.), 1512.15(C=N str.), 3040.22 (C-H str.), 1658.42 (C=O str.),1485.35 (C=N str.),3509.31 (-NH str.), 3312.27 (-NH₂ str.), 1022.37 (C-Br); ¹HNMR (ppm):δ 1.25 (4H methylene of pyrazoline), δ 4.80 (4H methylene side chain of pyrazoline), δ 3.62 (1H, dd, pyrazole ring); δ 1.54 (NH₂), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar-H), δ 7.58–7.72 (m, 2H, Ar-H).FAB Mass (m/z): 481.98 (Quasi-molecular ion peak (M+H)+).

Compound BR-4: 1-(5-(4-bromophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(hydrazinyl)propan-1-one

Molecular formula: C₁₈H₁₈BrN₅O₄; Molecular weight: 448.27; TLC (Rf value): 0.64; Element (Found/Calc.)%: Nitrogen (15.60/15.62); Oxygen (14.26/14.28); IR (cm⁻¹): 3208.26 (C-H str.)

1512.35(C=N str.), 3040.55(C-H str.), 1658.22 (C=O str.),1482.18 (C=N str.),3509.13 (-NH str.), 3312.50 (-NH₂ str.), 1022.27 (C-Br), 1569.25(N=O str.), 1365.53(N-O str.); ¹HNMR (ppm): δ 1.26 (4H methylene of pyrazoline), δ 4.82 (4H methylene side chain of pyrazoline), δ

3.62 (1H, dd, pyrazole ring); δ 1.54 (NH₂), 8.32 (N-H), δ 7.18–7.48 (m, 2H, Ar-H), δ 8.10–8.30 (m, 3H, Ar-H).FAB Mass (m/z): 447.05 (Quasi-molecular ion peak (M+H)+)

3.1 Antifungal Activity

In accordance with the present data obtained from antifungal activity, all the synthesized 1,3,5-trisubstituted pyrazole derivatives (ME1- ME8, CL1-CL8, BR1-BR4) have showed mild to good activity against tested organisms *Saccharomyces Cerevisiae*, *A. Niger* *C. Albicans*, *R. Oryzae*.

The Data of antifungal activity against the fungal strains (*Saccharomyces cerevisiae*, *A. niger*, *C. albicans*, *R. oryzae*) suggested the order of activity of compounds: BR-3 >BR-2 > BR-1 > CL-4 > BR-4 > CL-3 > CL-2 > ME-3> ME-2> CL-5 > CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1. Among these 1,3,5-trisubstituted pyrazole derivatives, compound CL-6 > ME-4 > ME-5 > ME-6 > ME-7 > CL-7 > CL-8>ME-8 >CL-1 >ME-1 shows mild activity and compounds CL-2, ME-3, ME-2 and CL-5 has showed moderate activity and BR-3 >BR-2 > BR-1 > CL-4 > BR-4 > CL-3 has shown best activity against all fungi strains (Table 1).

[A] Activity against *Sacharomyces Cerevisiae*:

The Compounds BR-3 (14.75±0.53; 17.65±0.83), BR-2 (12.45±0.28; 16.32±0.26), BR-1 (11.22±0.65; 15.25±0.74), CL-4 (11.72±0.34; 12.20±0.82), BR-4 (10.32±0.32; 12.25±0.45) and CL-3 (10.42±0.67 12.72±0.37) has shown zone of inhibition in mm in comparison to standard drug (Clotrimazole, 16±0.34; 21±0.24) has shown good activity against *Sacharomyces Cerevisiae* (Fungi strains) at 50µg concentration.

The Compounds BR-3 (17.65±0.83), BR-2 (16.32±0.26), BR-1 (15.25±0.74), CL-4 (12.20±0.82), CL-3 (12.72±0.37) and BR-4 (12.25±0.45) has shown zone of inhibition in comparison to standard drug (Clotrimazole, 21±0.24) has shown good activity against *Saccharomyces Cerevisiae* (Fungi strains) at 100µg concentration.

[B] Activity against *A. Niger*:

Compounds BR-3 (13.34±0.78); BR-2 (12.23±0.63); BR-4 (10.38±0.54); CL-4 (9.62±0.23) ;CL-3 (8.52±0.85); BR-1 (8.42±0.36) has shown zone of inhibition as compared to

standard drug (Clotrimazole, 15 ± 0.45) has shown good activity against *A. Niger* (Fungi strains) at 50 μ g concentration.

Compounds BR-3 (18.25 ± 0.32), BR-2 (20 ± 0.34), CL-4 (16.35 ± 0.65), BR-4 (14.42 ± 0.25);

(16.72 ± 0.68), CL-3 (14.32 ± 0.546), BR-1 (12.55 ± 0.42) has shown zone of inhibition comparison to standard drug (Clotrimazole, 20 ± 0.34) has shown good activity against *A. Niger* (Fungi strains) at 100 μ g concentration.

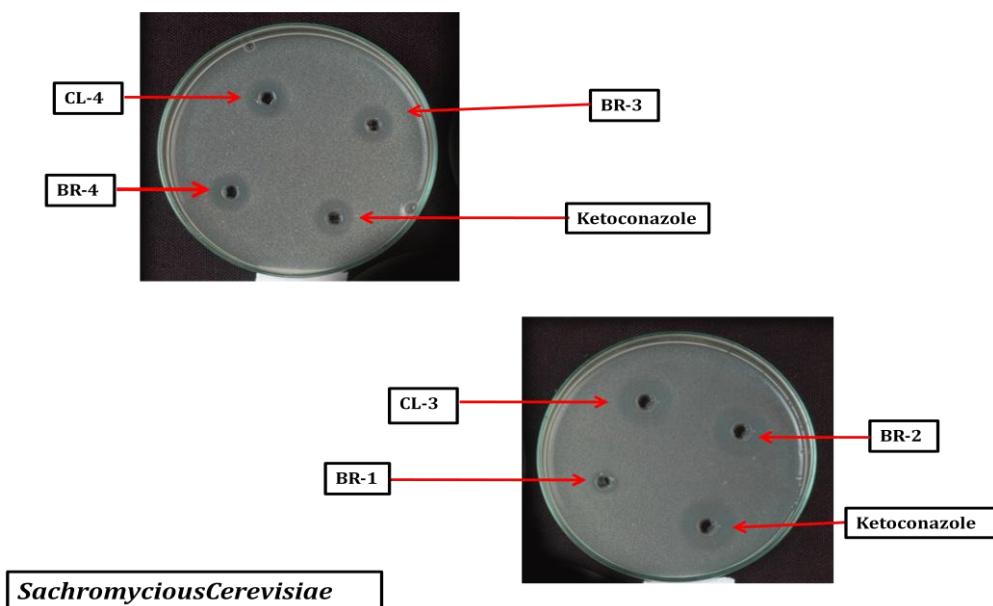


Fig. 1. Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

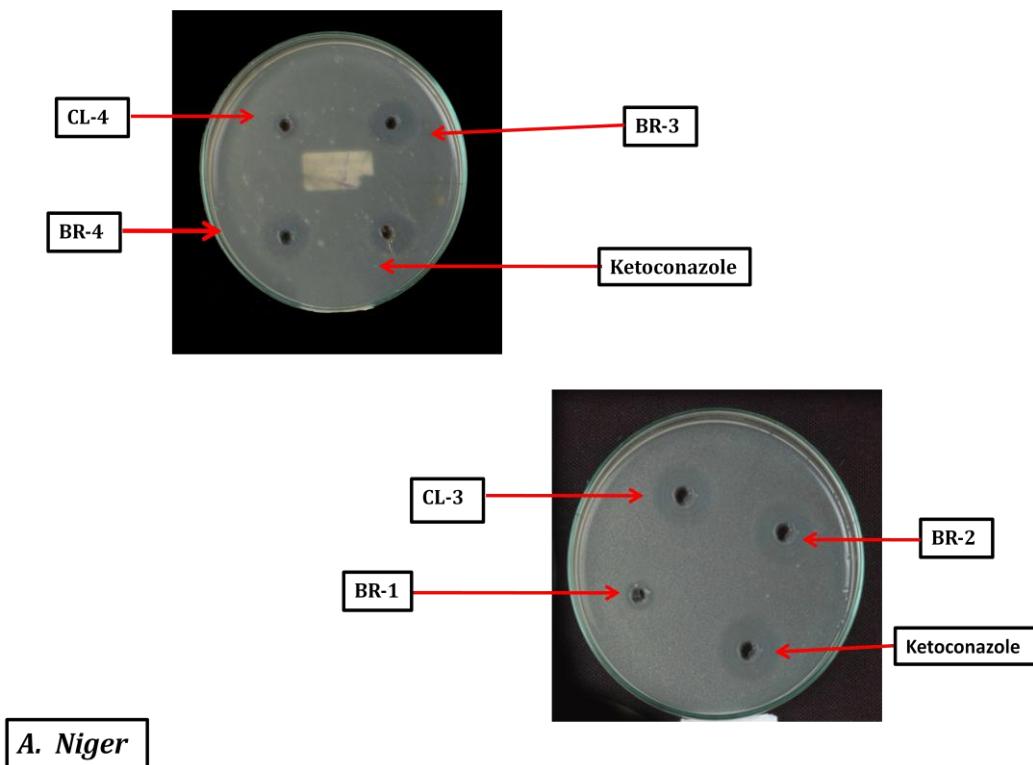
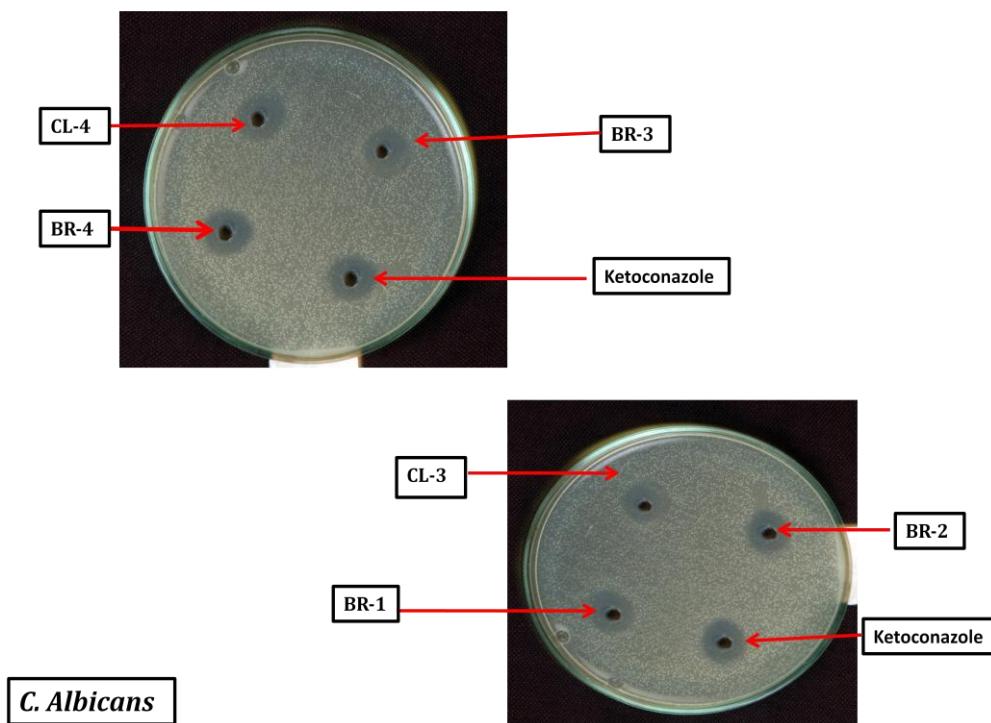


Fig. 2. Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

Table 1. Antifungal activity of synthesized pyrazole derivatives

COMPOUND	Zone of inhibition in mm							
	<i>Saccharomyces cerevisiae</i>		<i>A. niger</i>		<i>C. albicans</i>		<i>R. oryzae</i>	
Concentration	50	100	50	100	50	100	50	100
ME-1	2.32±0.33	3.22±0.52	3.32±0.63	5.52±0.73	5.22±0.83	6.32±0.42	7.35±0.62	9.25±0.33
ME-2	7.32±0.55	7.32±0.45	3.42±0.52	6.25±0.55	4.42±0.24	6.25±0.55	7.64±0.55	9.21±0.55
ME-3	8.32±0.33	8.72±0.64	4.52±0.76	7.64±0.42	5.23±0.52	8.54±0.33	7.35±0.23	9.42±0.63
ME-4	6.32±0.77	8.42±0.36	5.32±0.13	9.64±0.65	6.32±0.66	9.24±0.26	8.23±0.46	10.36±0.37
ME-5	5.32±0.22	6.62±0.53	2.62±0.37	5.12±0.33	4.52±0.33	6.56±0.42	7.16±0.55	9.32±0.74
ME-6	5.32±0.37	5.22±0.27	3.22±0.34	5.32±0.36	4.22±0.37	6.24±0.44	7.12±0.26	8.24±0.32
ME-7	4.32±0.14	5.72±0.72	3.32±0.57	5.22±0.73	4.42±0.72	6.36±0.26	7.34±0.32	8.23±0.46
ME-8	3.32±0.63	4.332±0.67	3.42±0.35	5.42±0.27	4.62±0.65	6.25±0.63	7.24±0.45	8.26±0.83
CL-1	2.52±0.45	3.62±0.25	3.62±0.44	5.32±0.54	4.32±0.73	6.32±0.38	7.32±0.63	8.32±0.87
CL-2	9.62±0.72	11.62±0.23	7.24±0.53	12.52±0.72	7.62±0.54	11.42±0.44	12.42±0.77	14.72±0.38
CL-3	10.42±0.67	12.72±0.37	8.52±0.85	14.32±0.546	10.32±0.36	14.62±0.33	13.72±0.24	16.32±0.53
CL-4	11.72±0.34	12.20±0.82	9.62±0.23	16.72±0.68	12.2±0.52	16.32±0.36	16.42±0.85	19.72±0.75
CL-5	07.32±0.88	8.32±0.44	6.65±0.36	10.52±0.82	5.62±0.26	9.52±0.41	9.62±0.43	11.62±0.22
CL-6	6.32±0.84	7.12±0.75	5.65±0.22	9.22±0.44	4.72±0.49	8.22±0.24	8.27±0.35	10.27±0.75
CL-7	04.22±0.26	5.52±0.32	3.62±0.55	5.32±0.53	4.32±0.23	6.32±0.43	7.32±0.72	8.32±0.87
CL-8	03.62±0.82	5.72±0.56	3.32±0.73	5.44±0.35	4.54±0.45	6.32±0.46	7.32±0.31	8.32±0.33
BR-1	11.22±0.65	15.25±0.74	8.42±0.36	12.55±0.42	8.28±0.22	11.44±0.33	12.56±0.54	15.66±0.25
BR-2	12.45±0.28	16.32±0.26	12.23±0.63	16.35±0.65	11.54±0.54	15.60±0.46	15.20±0.82	19.54±0.72
BR-3	14.75±0.53	17.65±0.83	13.34±0.78	18.25±0.32	13.20±0.35	17.52±0.54	17.65±0.77	21.05±0.31
BR-4	10.32±0.32	12.25±0.45	10.38±0.54	14.42±0.25	10.09±0.52	13.47±0.67	14.52±0.34	17.27±0.54
DMSO (Control)	-	-	-	-	-	-	-	-
Ketoconazole	15±0.32	20±0.26	14±0.32	19±0.23	13±0.45	18±0.69	18±0.30	22±0.33

**Fig. 3. Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*****[C] Activity against *C. albicans*:**

Compounds BR-3 (13.20±0.35), CL-4 (12.2±0.52), BR-2 (11.54±0.54), CL-3 (10.32±0.36), BR-4 (10.09±0.52) and BR-1

(8.28±0.22) has shown zone of inhibition comparison to standard drug (Clotrimazole, 14±0.46) has shown good activity against *C. albicans* (Fungi strains) at 50µg concentration.

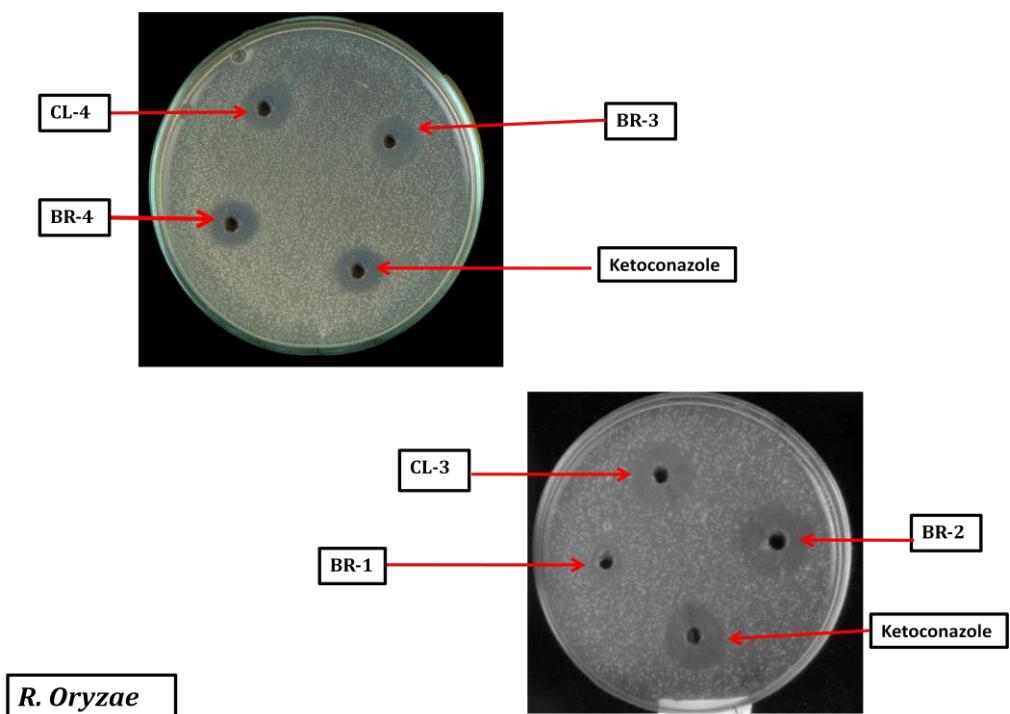


Fig. 4. Zone of inhibition of synthesized derivatives against *Staphylococcus aureus*

Compounds BR-3 (17.52 ± 0.54), CL-4 (16.32 ± 0.36), BR-2 (15.60 ± 0.46), CL-3 (14.62 ± 0.33), BR-4 (13.47 ± 0.67) and BR-1 (11.44 ± 0.33) has shown zone of inhibition comparison to standard drug (Clotrimazole, 19 ± 0.65) has shown good activity against *C. albicans* (Fungi strains) at $100 \mu\text{g}$ concentration.

[D] Activity against *R. oryzae*:

Compounds BR-3 (17.65 ± 0.77 ; CL-4 (16.42 ± 0.85); BR-2 (15.20 ± 0.82 ; BR-4 (14.52 ± 0.34); CL-3 (13.72 ± 0.24); BR-1 (12.56 ± 0.54) and CL-2 (14.52 ± 0.34) has shown zone of inhibition comparison to standard drug (Clotrimazole, 18 ± 0.30) has shown good activity against *R. Oryzae* (Fungi strains) at $50 \mu\text{g}$ concentration.

Compounds BR-3 (21.05 ± 0.31 ; CL-4 (19.72 ± 0.75); BR-2 (19.54 ± 0.72); BR-4 (17.27 ± 0.54); CL-3 (16.32 ± 0.53); BR-1 (15.66 ± 0.25) and CL-2 (22 ± 0.23) has shown zone of inhibition comparison to standard drug (Clotrimazole, 22 ± 0.33) has shown good activity against *R. oryzae* (Fungi strains) at $100 \mu\text{g}$ concentration. However, further studies on activity and long term toxicity are to be carried out before any conclusion are drawn, as these categories of drug are known to have potential antifungal activity. Testing on different models

can further substantiate the antifungal activity of the synthesized analogues. The graphical representation of zone of inhibition was shown in Figs. 1 to 4.

4. CONCLUSION

Using the Agar diffusion technique, all of the 2-pyrazolines were tested for antifungal activity against *Saccharomyces cerevisiae*, *A. niger*, *C. albicans*, and *R. oryzae*. The outcomes of this study were compared using ketoconazole as a benchmark. When pyrazoline was compared to standard, the antifungal activity results showed that the compounds exhibit considerable inhibitory effect on all fungal strains at both 50 g (0.05 ml) and 100 g (0.1 ml) dosing levels. Chemicals BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 had the highest activity of all the compounds examined. These compounds have halogens on the aromatic ring, indicating that electron withdrawing groups to the antifungal activity.

Electronegative groups (Br, Cl, F, and NO_2) must be present at the third and fifth positions of the 1,3,5-pyrazoline ring for significant antifungal action. The presence of an electronegative group (Br, Cl) at the third and fifth positions may be required for the best action against bacterial and fungal strains, however the addition of F, NO_2

has demonstrated moderate activity, while the replacement of -CH₃ -OCH₃ may reduce the activity. The synthesized compounds in the BR-1 through BR-4 class are the most active. This shows that bromine in the third and fifth positions of pyrazole is required for antifungal action, and that the presence of chloro-, bromo-, fluoro-, and nitrogen groups linked to the phenyl ring enhances antifungal activity. The antifungal activity data revealed that Cl, Br, F, and Nitro substitutions at the third and fifth positions may increase the antifungal activity of the compounds, whilst methyl and methoxy substitutions may decrease it.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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