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RESEARCH ARTICLE

Synthesis, Characterization, and Antifungal Evaluation of Some New 1,3,5-Trisubstituted Pyrazole Derivatives

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ABSTRACT

Objective: The objective of the paper was to design, synthesis, and characterization of new 1,3,5-trisubstituted-2-pyrazolines derivative and evaluate for antifungal activity. **Materials and Methods:** The 1,3,5-tri-substituted-2-pyrazolines derivatives have been synthesized by the reaction of chalcone derivatives with succinic hydrazide in the environment of pyridine. Total 20 compounds have been synthesized and characterized by the IR, 1H NMR, and mass spectral analysis. Antifungal activity of the compounds carried out on four fungal strains, that is, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Candida albicans*, and *Rhizopus oryzae* in two different concentrations, that is, 50 and 100 µg/ml by agar-diffusion method using cup-plate method and Ketoconazole was used as standard antifungal drug. **Results and Discussion:** In accordance with the data from antifungal activity, all the synthesized 1,3,5-trisubstituted pyrazole derivatives (ME1-ME8, CL1-CL8, and BR1-BR4) have shown mild to best activity against tested organisms. The data of antifungal activity against the fungal strains (*S. cerevisiae*, *A. niger*, *C. albicans*, and *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. The presence of electronegative group (Br, Cl, F, and NO_2) either at third and fifth position of 1,3,5-pyrazoline ring is required for the potent antifungal activity. The presence of electronegative group (Br, Cl) at third and fifth position may necessary for the best activity against bacterial and fungal strains but the addition of F, NO_2 has shown the moderate activity but in case of $-CH_3$ and $-OCH_3$ substitution may diminish the activity. The series BR-1 to BR-4 is most active compound of the synthesized compounds. **Conclusion:** The 1,3,5-trisubstituted pyrazole derivatives has been successfully synthesized and antifungal activity of the compounds denotes that the series BR-1 to BR-4 is most active compound of the all twenty synthesized compounds. The addition of electronegative group (Br, Cl) at third and fifth position in pyrazole ring may necessary for the activity against fungal strains.

Keywords: Agar diffusion, antifungal, cup-plate method, ketoconazole, pyrazole

INTRODUCTION

In modern era, the disease from the fungus as well as bacterial sources is exceeding drastically. The drugs which are used for the fungal infection treatment are ineffective by the times, the main reason behind is resistance by fungal strains. Hence, there is augment needs of new drugs or new chemical modified moieties that have to be effective against the bacterial and fungal infection. For searching the moieties, that have to be effective against fungal infection, we have find the diazoles that are reported to be used as antifungal agents.[1] An antifungal is used to treat fungal infections such as athlete's foot, ringworm, candidiasis (thrush), and serious systemic infections such as cryptococcal meningitis, and others. Antifungal works by exploiting differences between mammalian and

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fungal cells to kill off the fungal organism without dangerous effects on the host. Hence, searching of the new moieties that will helpful to treat fungal infection.[2]

Pyrazole derivatives have aroused considerable interest of chemists due to their versatile practical applications as well as their wide range of biochemical properties. Pyrazole has been reported to possess a broad spectrum of biological activities, namely, antifungal, anticancer, anti-inflammatory, antifungal, anti-proliferative, anticonvulsant, antioxidant, and antitubercular activities. Due to its wide range of biological activity pyrazole ring constitutes a relevant synthetic target in pharmaceutical industry.[3]

Marketed product that contains the pyrazole moieties are celecoxib, that is used as non-steroidal anti-inflammatory drug (NSAID) and used in the treatment of rheumatoid arthritis, osteoarthritis, acute pain, and menstruation. Phenazon (phenazon or antipyrine) is used as an analgesic and antipyretic; lonazolac is used as an NSAID; and betazole is a H_2 receptor agonist. It is used clinically to test gastric secretory function; fipronil is a broad spectrum insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride channels, components of the central nervous system.[4]

Chalcone is prepared by claisen-schmidt condensation of aromatic aldehyde and ketone by base catalyzed or acid catalyzed, followed by dehydration. The presence of α, β-unsaturated functional group is responsible for antifungal activity, which can be altered depending on the type of substituent present on the aromatic rings.[5] They also serve as a back bone for the synthesis of various heterocyclic compounds, as they undergo a variety of chemical reactions. Hence, chalcone play an important role in the synthesis medicinal compounds.[6] Literature review reveals that many chalcone derivatives of either natural or synthetic origin exhibit diverse pharmacological activities such as antifungal agents, antioxidant, anti-inflammatory activity, cytotoxic activity, hypo-glycemic activity, anti-hepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors, and antitumor activities.[7]

The combined synthesis reaction of chalcone with pyrazole moieties may be helpful to synthesize the new derivative of pyrazole that will be helpful for the searching the new derivatives as antifungal potential. The objective of the paper was to evaluate the efficacy of new 1,3,5-trisubstituted-2-pyrazolines derivative for their antifungal activity.

MATERIALS AND METHODS

Chemical p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone were purchased from HiMedia, New Delhi. Benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methyl benzaldehyde, and 4-methoxy benzaldehyde were purchased from Chemical Drug House, New Delhi, India. Succinic acid was purchased from Sigma-Aldrich, New Delhi. The chemical used for experimental work was synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on Bruker-alpha IR Spectrometer. Elemental analysis was performed and found values were within 0.4% of 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.

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 corresponding ester.
- Scheme III: Reaction of Succinic hydrazide with chalcone to form 1,3,5-trisubstituted pyrazole derivatives.

Scheme I: Synthesis of chalcones by claisen-schmidt condensation

Equimolar quantity (0.05 M) of substituted acetophenone was taken and mixed with equimolar quantity of benzaldehyde and substituted benzaldehyde. The mixture was dissolved in ethanol. The mixture was stirrer for 5 min and added 50% aqueous solution of potassium hydroxide was added slowly with continues stirring at room temperature for 24 h. The completion of the reaction was monitored by the TLC. Then, the reaction was completed, it poured into the crushed ice, solid product was obtained but if the solid product was not obtained so acidified with dilute hydrochloric acid.[5] 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 (0.05 M) can be easily converted to succinic hydrazide by reaction with hydrazine hydrate (0.05 M) in alcohol, then the reaction mixture was cooled to room temperature, succinic hydrazide separates as solid which was recrystallized using ethanol. The IR spectra denote the peak at 3500.66 (-NH str.); 3313.58 (NH_2 str.); 1658.32 (C=O); and 1430–3046.55 (CH-CH). The reaction was monitored by the TLC using hexane:ethyl acetate as mobile phase. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The synthesis is shown in Scheme II.

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

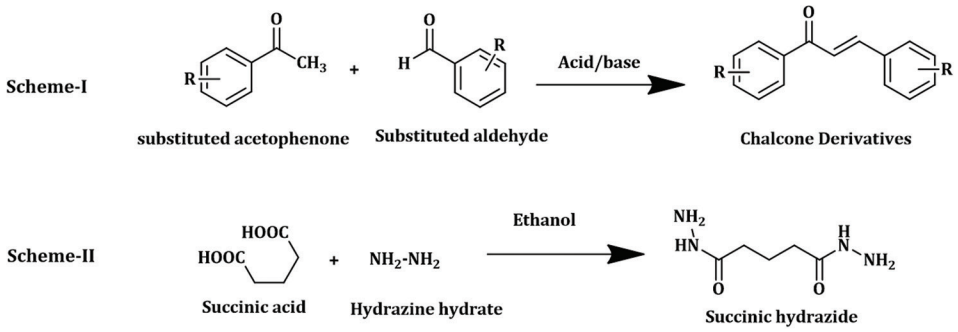
The synthesized chalcone derivatives with equimolar quantity (0.005 M) were mixed with succinic hydrazide (0.005 M) in absolute alcohol and addition of small amount of pyridine (0.01 M). The reaction mixture was refluxed at 65°C up to 2–6 h. The reaction was monitored by the TLC using ethyl acetate:hexane as mobile phase. The solvent was completely evaporated and then was poured into the ice cold water with constant stirring, that convert liquid into solid product, that resulted into the corresponding synthesized product.[8] This solid was filtered under vacuum and dried. The synthesized compound purified by the column chromatography was obtained as pale yellow solid color powder. The synthesis is shown in Scheme III.

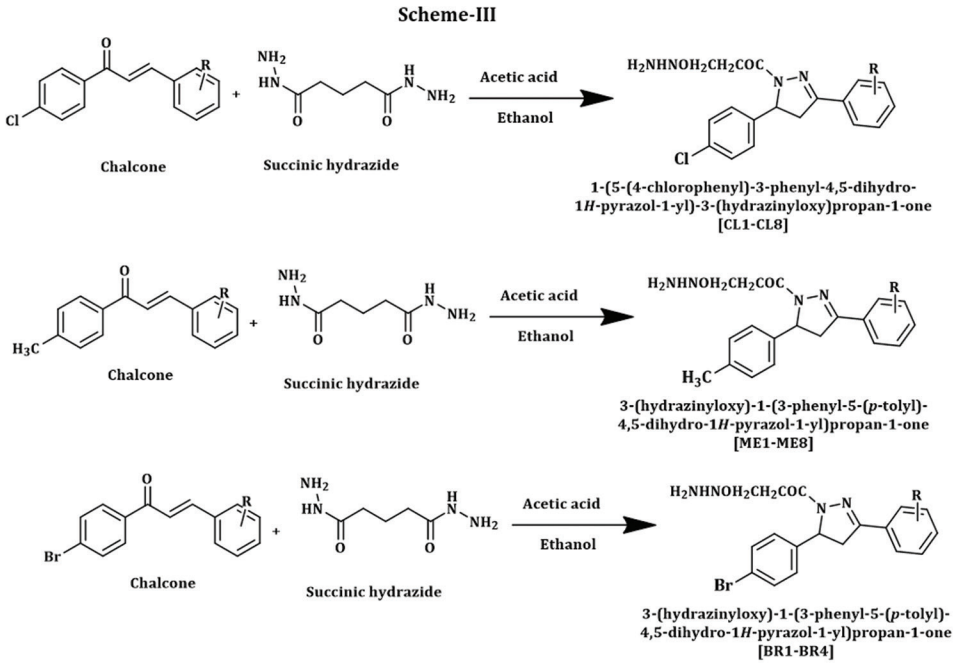
Evaluation of antifungal activity

To study and evaluate antifungal activity, there should be reliable and reproducible methods to evaluate them. Agar diffusion method was used for evaluation of the antifungal activity using cup-plate method.[9-12]

Antifungal screening of the synthesized compounds

All synthesized 20 compounds were screened for their antifungal activity. The fungi employed for the screening were *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans* and were procured from IMTECH Chandigarh. Ketoconazole was employed as standard. The test organisms were sub-cultured using potato-dextrose agar (PDA) medium.[13-16] 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. The activity of the derivatives was performed by cup-plate method at different concentration levels. Ketoconazole was used as standard drug. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml, Analar grade) to give a concentration of 1000 g/ml. The solutions of each test compound, control, and reference standards (0.05 ml and 0.1 ml) were added separately in the cups and the plates were kept undisturbed for at least 2 h in a refrigerator to allow diffusion of the solution properly into the PDA medium. Petri dishes were subsequently kept at room temperature for 48 h. 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.

RESULTS AND DISCUSSION

Scheme I

The synthesized compounds were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The IR spectra denote 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 (OCH_3). These compounds 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 were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The IR spectra denote 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 (-NH_2 str), 852.22 (C-Cl), 1025.27 (C-Br), 1118.62 (C-F), 1072.46 (C-OCH_3), 1569 (N=O str.), and 1365 (N-O str.). These compounds 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 (NH_2), 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: $C_{19}H_{22}N_4O_2$; 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 C_6H_5, 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH_2 str.); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{22}H_{20}ClN_3O_4S$; 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 (-NH_2 str.), 1118.62 (C-F); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}ClN_4O_2$; 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); 1H NMR: 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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}BrN_4O_2$; 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 (-NH_2 str.), 1020.27 (C-Br); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}N_5O_4$; 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 (-NH_2 str.), 1562.25 (N=O str,), 1362.42 (N-O str.); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{20}H_{24}N_4O_2$; molecular weight: 352.43; TLC (Rf value): 0.64; element (Found/Calc.)%: Nitrogen (9.58/9.60); sulfur (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 (-NH_2 str.); 1H NMR (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 (NH_2), 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 (Quasi-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 (-NH_2 str.), 1074.26 (-OCH_3);1H NMR (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 (Quasi-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: $C_{21}H_{27}N_5O_2$; 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 (-NH_2 str.); 1H NMR (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 (NH_2), 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 ($N(CH_3)_2$). FAB Mass (m/z): 381.47 (Quasi-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: $C_{18}H_{19}ClN_4O_2$; 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-C_6H_5, 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 (-NH_2 str.), 852.22 (C-Cl); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{18}H_{18}ClFN_4O_2$; 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 (-NH_2 str.). 850.22 (C-Cl), 1118.62 (C-F); 1H NMR (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 (NH_2), 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 (Quasi-molecular ion peak (M+H)+).

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

Molecular formula: $C_{18}H_{18}Cl_2N_4O_2$; 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 (-NH_2 str.), 852.22 (C-Cl); 1H NMR (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 (NH_2), 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: $C_{18}H_{18}BrClN_4O_2$; 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 ($-NH_2$ str.), 850.12 (C-Cl), 1020.37 (C-Br); 1H NMR(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 (NH_2), 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: $C_{18}H_{18}ClN_5O_4$; 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.); 1H NMR (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: $C_{19}H_{21}ClN_4O_2$; 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 ($-NH_2$ str.). 850.22 (C-Cl); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}ClN_4O_3$; 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 ($-NH_2$ str.), 850.22 (C-Cl str.), 1072.46 ($-OCH_3$); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{20}H_{24}ClN_5O_2$; 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 ($-NH_2$ str.), 850.22 (C-Cl); 1HMNR (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 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 6.65–7.50 (m, 2H, Ar–H), 2.58 ($N(CH_3)_2$). FAB Mass (m/z): 401.16 (Quasi-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: $C_{18}H_{18}BrFN_4O_2$; 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 (-NH_2 str.), 1025.27 (C-Br), 1118.62 (C-F); 1H NMR (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 (NH_2), 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-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrClN_4O_2$; 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 (-NH_2 str.), 1028.22 (C-Br), 850.25 (C-Cl); 1H NMR (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 (NH_2), 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-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{18}H_{18}Br_2N_4O_2$; 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 (-NH_2 str.), 1022.37 (C-Br); 1H NMR (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 (NH_2), 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-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrN_5O_4$; 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 (-NH_2 str.), 1022.27 (C-Br), 1569.25 (N=O str.), 1365.53 (N-O str.); 1H NMR (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 (NH_2), 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)+).

ANTIFUNGAL ACTIVITY

In accordance with the 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 *S. cerevisiae*, *A. niger C. albicans*, and *R. Oryzae*.

The data of antifungal activity against the fungal strains (*S. cerevisiae*, *A. niger*, *C. albicans*, and *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 have showed moderate activity and BR-3 > BR-2 > BR-1 > CL-4 > BR-4 > CL-3 have shown best activity against all fungi strains [Table 1].

[A] Activity against *S. 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

Table 1: Antifungal activity of synthesized pyrazole derivatives

Compound	Zone of inhibition in mm							
	Saccharomyces cerevisiae		*Niger*		*Candida albicans*		*Rhizopus oryzae*	
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

(10.42 ± 0.67; 12.72 ± 0.37) have shown zone of inhibition in mm in comparison to standard drug (Clotrimazole, 16 ± 0.34; 21 ± 0.24) and have shown good activity against *S. cerevisiae* (Fungi strains) at 50 µg 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) have shown zone of inhibition in comparison to standard drug (Clotrimazole, 21 ± 0.24) and have shown good activity against *S. cerevisiae* (Fungi strains) at 100 µg 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); and BR-1 (8.42 ± 0.36) have shown zone of inhibition as compared to standard drug (Clotrimazole, 15 ± 0.45) and have shown good activity against *A. niger* (Fungi strains) at 50 µg concentration.

Compounds BR-3 (18.25 ± 0.32), BR-2 (16.35 ± 0.65), BR-4 (14.42 ± 0.25); Cl-4 (16.72 ± 0.68), CL-3 (14.32 ± 0.546), and BR-1 (12.55 ± 0.42) have shown zone of inhibition comparison to standard drug (Clotrimazole, 20 ± 0.34) and have shown good activity against *A. niger* (Fungi strains) at 100 µg concentration.

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

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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 19 ± 0.65) and have shown

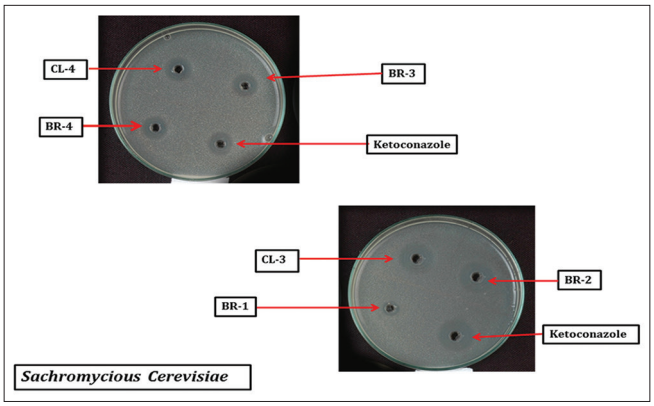


Figure 1: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

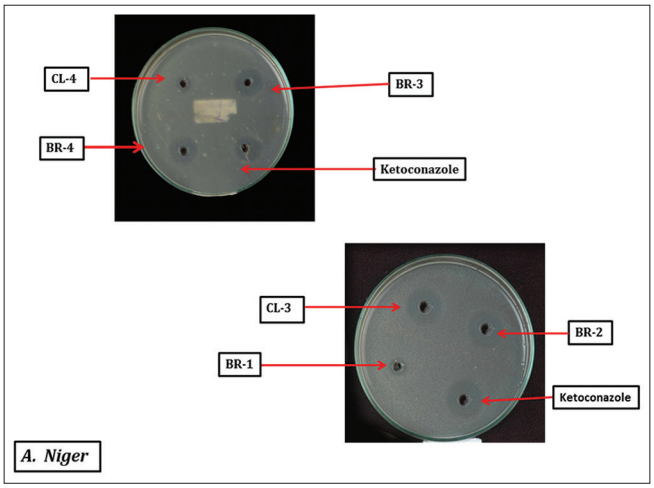


Figure 2: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

good activity against *C. Albicans* (Fungi strains) at 100 µg 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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 18 ± 0.30) and have shown good activity against *R. oryzae* (Fungi strains) at 50 µg 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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 22 ± 0.33) and have shown good activity against *R. oryzae* (Fungi strains) at 100 µg concentration. However, further studies on

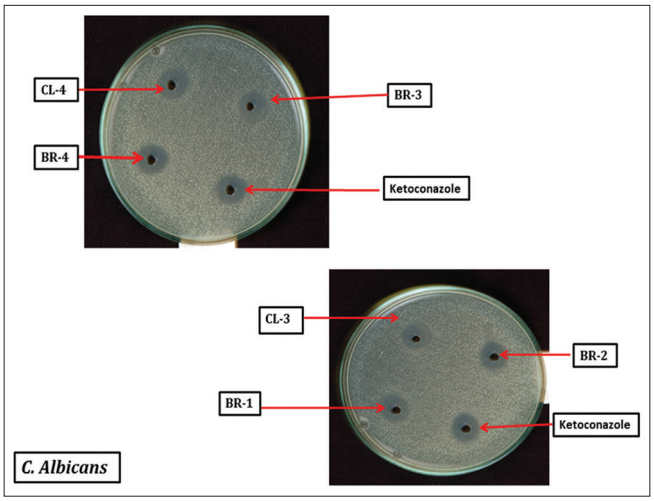


Figure 3: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

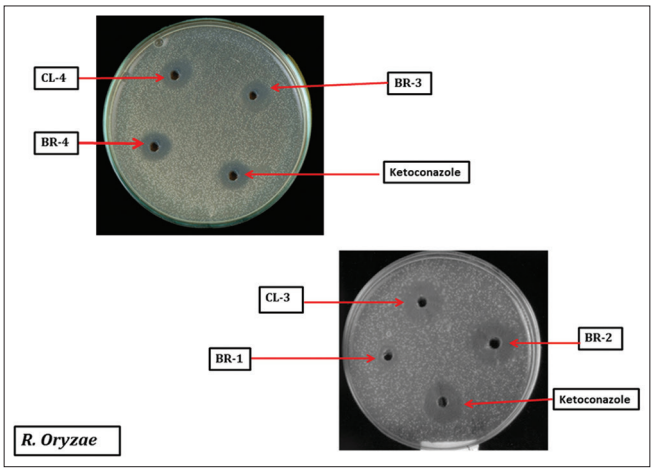


Figure 4: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

activity and long-term toxicity are to be carried out before any conclusion is 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 is shown in Figures 1-4.

CONCLUSION

All the 2-pyrazolines have been evaluated for their antifungal activity against *S. cerevisiae*, *A. niger C. albicans*, and *R. oryzae* using Agar diffusion method. The results of this evaluation have been compared by taking ketoconazole as standard. The antifungal activity data of pyrazoline indicated that the

compounds have significant inhibitory activity on all the fungal strains at both 50 μg (0.05 ml) and 100 μg (0.1 ml) dose levels when compared with standard. Among all the compounds tested, compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 possessed maximum activity. These compounds possessed the halogens on the aromatic ring and thus revealed the positive contribution of electron withdrawing groups to the antifungal activity.

The presence of electronegative group (Br, Cl, F, and NO_2) either at third and fifth position of 1,3,5-pyrazoline ring is required for the potent antifungal activity. The presence of electronegative group (Br, Cl) at third and fifth position may necessary for the best activity against bacterial and fungal strains but the addition of F, NO_2 has shown the moderate activity but in case of -CH_3 -OCH_3 substitution may diminish the activity.

The series BR-1 to BR-4 is most active compound of the synthesized compounds. This evident that the presence of bromine in the third and fifth position of pyrazole is essential for the antifungal activity and chloro, bromo, fluoro, and nitro group attached at phenyl ring enhance the antifungal activity. The result data of antifungal activity suggested that Cl, Br, F, and Nitro substitution at third and fifth position may enhance the antifungal activity of the compounds but the methyl and methoxy substitution may resulted in reduction of the activity.

ACKNOWLEDGMENT

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CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest

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fungal cells to kill off the fungal organism without dangerous effects on the host. Hence, searching of the new moieties that will helpful to treat fungal infection.[2]

Pyrazole derivatives have aroused considerable interest of chemists due to their versatile practical applications as well as their wide range of biochemical properties. Pyrazole has been reported to possess a broad spectrum of biological activities, namely, antifungal, anticancer, anti-inflammatory, antifungal, anti-proliferative, anticonvulsant, antioxidant, and antitubercular activities. Due to its wide range of biological activity pyrazole ring constitutes a relevant synthetic target in pharmaceutical industry.[3]

Marketed product that contains the pyrazole moieties are celecoxib, that is used as non-steroidal anti-inflammatory drug (NSAID) and used in the treatment of rheumatoid arthritis, osteoarthritis, acute pain, and menstruation. Phenazon (phenazon or antipyrine) is used as an analgesic and antipyretic; lonazolac is used as an NSAID; and betazole is a H_2 receptor agonist. It is used clinically to test gastric secretory function; fipronil is a broad spectrum insecticide that disrupts the insect central nervous system by blocking the passage of chloride ions through the GABA receptor and glutamate-gated chloride channels, components of the central nervous system.[4]

Chalcone is prepared by claisen-schmidt condensation of aromatic aldehyde and ketone by base catalyzed or acid catalyzed, followed by dehydration. The presence of α, β-unsaturated functional group is responsible for antifungal activity, which can be altered depending on the type of substituent present on the aromatic rings.[5] They also serve as a back bone for the synthesis of various heterocyclic compounds, as they undergo a variety of chemical reactions. Hence, chalcone play an important role in the synthesis medicinal compounds.[6] Literature review reveals that many chalcone derivatives of either natural or synthetic origin exhibit diverse pharmacological activities such as antifungal agents, antioxidant, anti-inflammatory activity, cytotoxic activity, hypo-glycemic activity, anti-hepatotoxic, antimalarial, antileishmanial, tyrosine inhibitors, and antitumor activities.[7]

The combined synthesis reaction of chalcone with pyrazole moieties may be helpful to synthesize the new derivative of pyrazole that will be helpful for the searching the new derivatives as antifungal potential. The objective of the paper was to evaluate the efficacy of new 1,3,5-trisubstituted-2-pyrazolines derivative for their antifungal activity.

MATERIALS AND METHODS

Chemical p-chloroacetophenone, p-bromoacetophenone, and p-methylacetophenone were purchased from HiMedia, New Delhi. Benzaldehyde, 4-fluorobenzaldehyde, 4-chlorobenzaldehyde, 4-bromobenzaldehyde, 4-nitrobenzaldehyde, 4-methyl benzaldehyde, and 4-methoxy benzaldehyde were purchased from Chemical Drug House, New Delhi, India. Succinic acid was purchased from Sigma-Aldrich, New Delhi. The chemical used for experimental work was synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. IR spectra were recorded on Bruker-alpha IR Spectrometer. Elemental analysis was performed and found values were within 0.4% of 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.

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 corresponding ester.
- Scheme III: Reaction of Succinic hydrazide with chalcone to form 1,3,5-trisubstituted pyrazole derivatives.

Scheme I: Synthesis of chalcones by claisen-schmidt condensation

Equimolar quantity (0.05 M) of substituted acetophenone was taken and mixed with equimolar quantity of benzaldehyde and substituted benzaldehyde. The mixture was dissolved in ethanol. The mixture was stirrer for 5 min and added 50% aqueous solution of potassium hydroxide was added slowly with continues stirring at room temperature for 24 h. The completion of the reaction was monitored by the TLC. Then, the reaction was completed, it poured into the crushed ice, solid product was obtained but if the solid product was not obtained so acidified with dilute hydrochloric acid.[5] 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 (0.05 M) can be easily converted to succinic hydrazide by reaction with hydrazine hydrate (0.05 M) in alcohol, then the reaction mixture was cooled to room temperature, succinic hydrazide separates as solid which was recrystallized using ethanol. The IR spectra denote the peak at 3500.66 (-NH str.); 3313.58 (NH_2 str.); 1658.32 (C=O); and 1430–3046.55 (CH-CH). The reaction was monitored by the TLC using hexane:ethyl acetate as mobile phase. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The synthesis is shown in Scheme II.

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

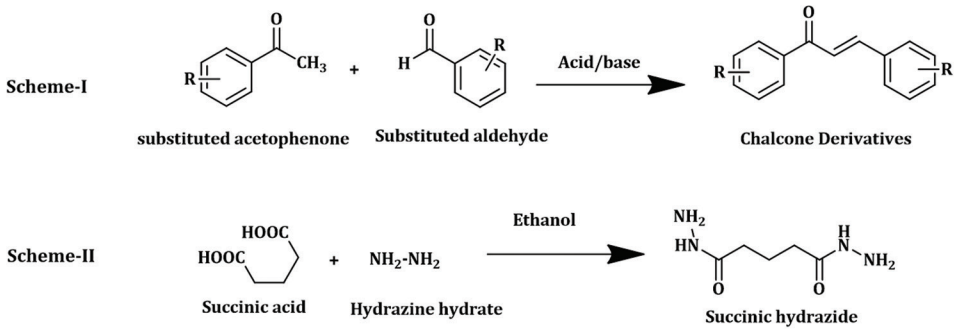
The synthesized chalcone derivatives with equimolar quantity (0.005 M) were mixed with succinic hydrazide (0.005 M) in absolute alcohol and addition of small amount of pyridine (0.01 M). The reaction mixture was refluxed at 65°C up to 2–6 h. The reaction was monitored by the TLC using ethyl acetate:hexane as mobile phase. The solvent was completely evaporated and then was poured into the ice cold water with constant stirring, that convert liquid into solid product, that resulted into the corresponding synthesized product.[8] This solid was filtered under vacuum and dried. The synthesized compound purified by the column chromatography was obtained as pale yellow solid color powder. The synthesis is shown in Scheme III.

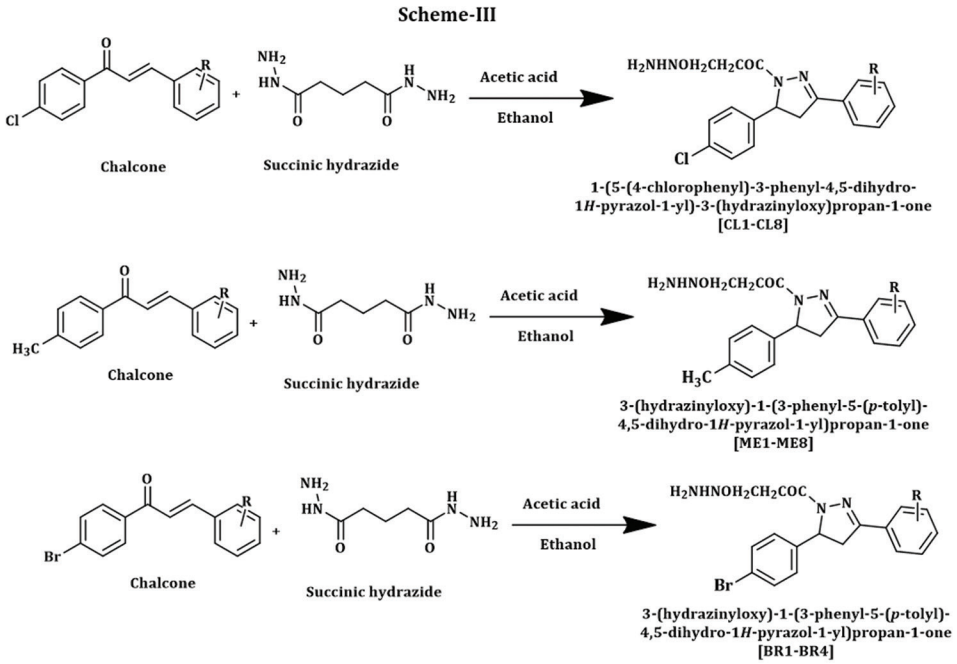
Evaluation of antifungal activity

To study and evaluate antifungal activity, there should be reliable and reproducible methods to evaluate them. Agar diffusion method was used for evaluation of the antifungal activity using cup-plate method.[9-12]

Antifungal screening of the synthesized compounds

All synthesized 20 compounds were screened for their antifungal activity. The fungi employed for the screening were *Saccharomyces cerevisiae*, *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans* and were procured from IMTECH Chandigarh. Ketoconazole was employed as standard. The test organisms were sub-cultured using potato-dextrose agar (PDA) medium.[13-16] 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. The activity of the derivatives was performed by cup-plate method at different concentration levels. Ketoconazole was used as standard drug. Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml, Analar grade) to give a concentration of 1000 g/ml. The solutions of each test compound, control, and reference standards (0.05 ml and 0.1 ml) were added separately in the cups and the plates were kept undisturbed for at least 2 h in a refrigerator to allow diffusion of the solution properly into the PDA medium. Petri dishes were subsequently kept at room temperature for 48 h. 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.

RESULTS AND DISCUSSION

Scheme I

The synthesized compounds were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The IR spectra denote 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 (OCH_3). These compounds 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 were characterized by the infrared spectroscopy and proton NMR spectroscopy and were found reliable with probable structure. Obtained compounds were characterized by IR, 1H NMR and were found consistent with an expected structure. The IR spectra denote 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 (-NH_2 str), 852.22 (C-Cl), 1025.27 (C-Br), 1118.62 (C-F), 1072.46 (C-OCH_3), 1569 (N=O str.), and 1365 (N-O str.). These compounds 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 (NH_2), 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: $C_{19}H_{22}N_4O_2$; 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 C_6H_5, 3042.55 (C-H str.), 1660.32 (C=O str.), 1486.20 (C=N str.), 3502.21 (-NH str.), 3315.50 (-NH_2 str.); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{22}H_{20}ClN_3O_4S$; 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 (-NH_2 str.), 1118.62 (C-F); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}ClN_4O_2$; 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); 1H NMR: 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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}BrN_4O_2$; 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 (-NH_2 str.), 1020.27 (C-Br); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}N_5O_4$; 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 (-NH_2 str.), 1562.25 (N=O str,), 1362.42 (N-O str.); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{20}H_{24}N_4O_2$; molecular weight: 352.43; TLC (Rf value): 0.64; element (Found/Calc.)%: Nitrogen (9.58/9.60); sulfur (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 (-NH_2 str.); 1H NMR (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 (NH_2), 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 (Quasi-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 (-NH_2 str.), 1074.26 (-OCH_3);1H NMR (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 (Quasi-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: $C_{21}H_{27}N_5O_2$; 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 (-NH_2 str.); 1H NMR (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 (NH_2), 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 ($N(CH_3)_2$). FAB Mass (m/z): 381.47 (Quasi-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: $C_{18}H_{19}ClN_4O_2$; 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-C_6H_5, 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 (-NH_2 str.), 852.22 (C-Cl); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{18}H_{18}ClFN_4O_2$; 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 (-NH_2 str.). 850.22 (C-Cl), 1118.62 (C-F); 1H NMR (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 (NH_2), 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 (Quasi-molecular ion peak (M+H)+).

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

Molecular formula: $C_{18}H_{18}Cl_2N_4O_2$; 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 (-NH_2 str.), 852.22 (C-Cl); 1H NMR (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 (NH_2), 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: $C_{18}H_{18}BrClN_4O_2$; 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 ($-NH_2$ str.), 850.12 (C-Cl), 1020.37 (C-Br); 1H NMR(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 (NH_2), 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: $C_{18}H_{18}ClN_5O_4$; 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.); 1H NMR (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: $C_{19}H_{21}ClN_4O_2$; 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 ($-NH_2$ str.). 850.22 (C-Cl); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{19}H_{21}ClN_4O_3$; 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 ($-NH_2$ str.), 850.22 (C-Cl str.), 1072.46 ($-OCH_3$); 1H NMR (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 (NH_2), 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 (Quasi-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: $C_{20}H_{24}ClN_5O_2$; 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 ($-NH_2$ str.), 850.22 (C-Cl); 1HMNR (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 (NH_2), 8.32 (N-H), δ 7.30–7.48 (m, 2H, Ar–H), δ 6.65–7.50 (m, 2H, Ar–H), 2.58 ($N(CH_3)_2$). FAB Mass (m/z): 401.16 (Quasi-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: $C_{18}H_{18}BrFN_4O_2$; 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 (-NH_2 str.), 1025.27 (C-Br), 1118.62 (C-F); 1H NMR (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 (NH_2), 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-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrClN_4O_2$; 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 (-NH_2 str.), 1028.22 (C-Br), 850.25 (C-Cl); 1H NMR (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 (NH_2), 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-(hydrazinyloxy) propan-1-one

Molecular formula: $C_{18}H_{18}Br_2N_4O_2$; 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 (-NH_2 str.), 1022.37 (C-Br); 1H NMR (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 (NH_2), 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-(hydrazinyloxy)propan-1-one

Molecular formula: $C_{18}H_{18}BrN_5O_4$; 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 (-NH_2 str.), 1022.27 (C-Br), 1569.25 (N=O str.), 1365.53 (N-O str.); 1H NMR (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 (NH_2), 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)+).

ANTIFUNGAL ACTIVITY

In accordance with the 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 *S. cerevisiae*, *A. niger C. albicans*, and *R. Oryzae*.

The data of antifungal activity against the fungal strains (*S. cerevisiae*, *A. niger*, *C. albicans*, and *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 have showed moderate activity and BR-3 > BR-2 > BR-1 > CL-4 > BR-4 > CL-3 have shown best activity against all fungi strains [Table 1].

[A] Activity against *S. 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

Table 1: Antifungal activity of synthesized pyrazole derivatives

Compound	Zone of inhibition in mm							
	Saccharomyces cerevisiae		*Niger*		*Candida albicans*		*Rhizopus oryzae*	
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

(10.42 ± 0.67; 12.72 ± 0.37) have shown zone of inhibition in mm in comparison to standard drug (Clotrimazole, 16 ± 0.34; 21 ± 0.24) and have shown good activity against *S. cerevisiae* (Fungi strains) at 50 µg 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) have shown zone of inhibition in comparison to standard drug (Clotrimazole, 21 ± 0.24) and have shown good activity against *S. cerevisiae* (Fungi strains) at 100 µg 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); and BR-1 (8.42 ± 0.36) have shown zone of inhibition as compared to standard drug (Clotrimazole, 15 ± 0.45) and have shown good activity against *A. niger* (Fungi strains) at 50 µg concentration.

Compounds BR-3 (18.25 ± 0.32), BR-2 (16.35 ± 0.65), BR-4 (14.42 ± 0.25); Cl-4 (16.72 ± 0.68), CL-3 (14.32 ± 0.546), and BR-1 (12.55 ± 0.42) have shown zone of inhibition comparison to standard drug (Clotrimazole, 20 ± 0.34) and have shown good activity against *A. niger* (Fungi strains) at 100 µg concentration.

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

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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 19 ± 0.65) and have shown

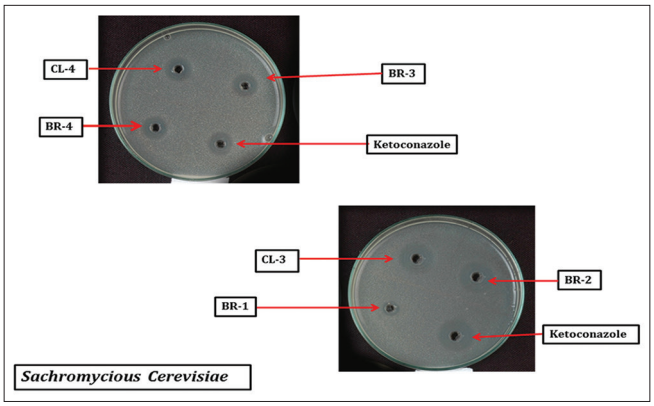


Figure 1: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

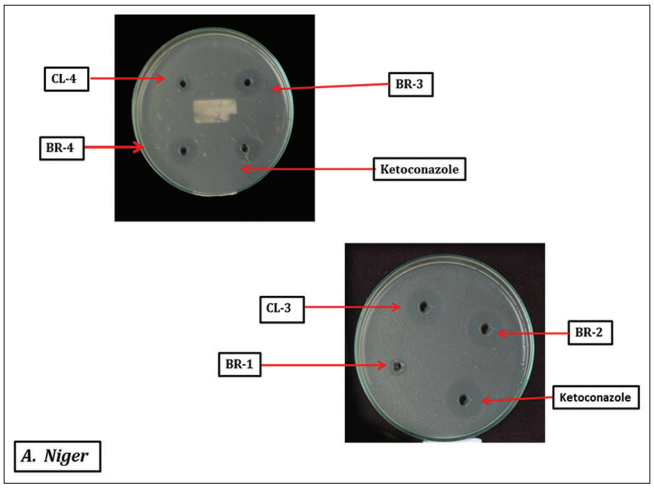


Figure 2: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

good activity against *C. Albicans* (Fungi strains) at 100 µg 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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 18 ± 0.30) and have shown good activity against *R. oryzae* (Fungi strains) at 50 µg 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) have shown zone of inhibition comparison to standard drug (Clotrimazole, 22 ± 0.33) and have shown good activity against *R. oryzae* (Fungi strains) at 100 µg concentration. However, further studies on

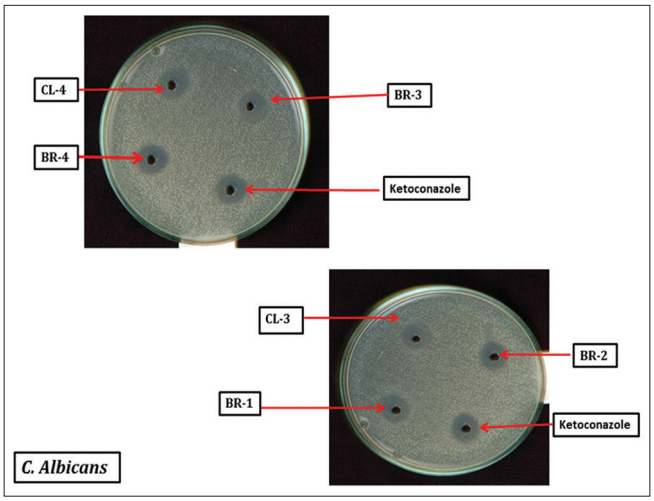


Figure 3: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

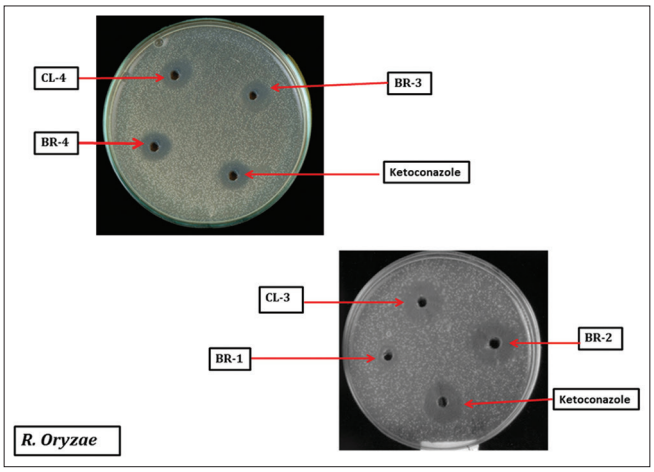


Figure 4: Zone of inhibition of synthesized derivatives against Staphylococcus aureus

activity and long-term toxicity are to be carried out before any conclusion is 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 is shown in Figures 1-4.

CONCLUSION

All the 2-pyrazolines have been evaluated for their antifungal activity against *S. cerevisiae*, *A. niger C. albicans*, and *R. oryzae* using Agar diffusion method. The results of this evaluation have been compared by taking ketoconazole as standard. The antifungal activity data of pyrazoline indicated that the

compounds have significant inhibitory activity on all the fungal strains at both 50 μg (0.05 ml) and 100 μg (0.1 ml) dose levels when compared with standard. Among all the compounds tested, compounds BR-3, BR-2, BR-1, CL-4, BR-4, and CL-3 possessed maximum activity. These compounds possessed the halogens on the aromatic ring and thus revealed the positive contribution of electron withdrawing groups to the antifungal activity.

The presence of electronegative group (Br, Cl, F, and NO_2) either at third and fifth position of 1,3,5-pyrazoline ring is required for the potent antifungal activity. The presence of electronegative group (Br, Cl) at third and fifth position may necessary for the best activity against bacterial and fungal strains but the addition of F, NO_2 has shown the moderate activity but in case of -CH_3 -OCH_3 substitution may diminish the activity.

The series BR-1 to BR-4 is most active compound of the synthesized compounds. This evident that the presence of bromine in the third and fifth position of pyrazole is essential for the antifungal activity and chloro, bromo, fluoro, and nitro group attached at phenyl ring enhance the antifungal activity. The result data of antifungal activity suggested that Cl, Br, F, and Nitro substitution at third and fifth position may enhance the antifungal activity of the compounds but the methyl and methoxy substitution may resulted in reduction of the activity.

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CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest

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