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Microwave Assisted Synthesis and Biological Evaluation of Indazole Derivatives

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KEYWORDS

Indazole,
Microwave,
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ABSTRACT: In this study, we synthesized indazole derivatives, including 1-H indazole and 4-chloro-1-H indazole, using microwave-assisted reactions with ortho-chlorobenzaldehyde, ortho-nitro benzaldehyde, and 2,6-dichloro benzaldehyde as starting materials. The reactions were carried out in distilled water, offering a fast and efficient synthesis process compared to conventional methods. The products were characterized through melting point determination, thin-layer chromatography (TLC), infrared (IR) spectroscopy, and nuclear magnetic resonance (NMR) spectroscopy, confirming their structures. The antifungal activity of the compounds was tested against Candida albicans, showing promising results. This microwave-assisted approach provides a convenient and effective way to synthesize indazole derivatives with potential antifungal properties.

Introduction: Indazole is a fused bicyclic compound consisting of a benzene and pyrazole ring, with 1H-indazole being its more stable tautomer. It can act as both an acid and a base, with pKa values of 1.04 and 13.86, making it reactive in different pH environments. First synthesized by Hermann Emil Fischer in 1883, indazole has since gained importance in medicinal chemistry due to its diverse biological activities, including anti-inflammatory and antibacterial properties. Microwave-assisted synthesis has made its production more efficient by reducing reaction times, increasing yields, and minimizing waste. This method relies on dielectric heating, where polar molecules absorb microwave energy, generating rapid and uniform heat, leading to better reaction control and sustainability.

Objectives: This study focuses on efficiently synthesizing and analyzing indazole derivatives using microwave-assisted methods. It also examines their antifungal potential against Candida albicans to explore possible medical applications.

Methods: Indazole derivatives were synthesized using an eco-friendly ultrasonic irradiation method in distilled water. A reaction mixture containing hydrazine hydrate (2 mmol), LPP (10%), and different benzaldehyde derivatives (1 mmol)—o-chlorobenzaldehyde (for 2a), o-nitro benzaldehyde (for 2b), and 2,6-dichloro benzaldehyde (for 2c)—was exposed to 425 MW ultrasonic irradiation for 18 minutes. The progress was tracked using TLC (n-hexane: ethyl acetate, 5:5). After completion, the reaction mixture was diluted with hot ethanol, filtered, and the catalyst was washed with ethanol (three times, 5 mL each). The crude product was then collected, concentrated, and purified through recrystallization in ethanol.

Results: The synthesis yielded 77%, 81.5%, and 85.79% for compounds 2a, 2b, and 2c, respectively. The melting points of compounds 2a, 2b, and 2c were recorded as 145°C, 145°C, and 153°C, respectively. Their Rf values, determined using TLC, were found to be 0.76 for 2a, 0.69 for 2b, and 0.589 for 2c. IR and NMR spectroscopy confirmed the successful formation of indazole derivatives. The antifungal study on Candida albicans revealed that compound 2c showed the highest activity.

Conclusions: A straightforward and highly effective microwave-assisted synthesis approach has been developed. This method allows for the successful production of indazole derivatives with high yields and reduced reaction times. It has been shown to be environmentally friendly, making it a practical option for creating new indazole derivatives for potential antifungal applications.

1. Introduction

1.1 Indazole and its derivatives:

Indazole is a two-ring compound made up of a benzene ring and a pyrazole ring that are connected. It typically exists in two different forms, known as tautomers: 1H-indazole and 2H-indazole. Among these, 1H-indazole is more stable under normal conditions. This compound displays amphoteric characteristics, meaning it can function as both an acid and a base. Its pKa values are around 1.04 when it gains a proton and 13.86 when it loses one, reflecting its ability to react in different pH environments. [1]

In 1883, German chemist Hermann Emil Fischer synthesized indazole by heating ortho-hydrazine cinnamic acid, leading to the formation of this unique structure. This discovery expanded the understanding of heterocyclic compounds and opened new avenues for research. [2]

While indazole is rare in nature, it has been identified in certain alkaloids from plants like Nigella sativa (black cumin). These naturally occurring indazoles possess unique structures and potential therapeutic properties. [3]

The indazole scaffold is highly valued for its versatility in modern medicinal chemistry. Derivatives of indazole have demonstrated a wide range of biological activities, including anti-inflammatory, antibacterial, antifungal, and antitumor effects. This versatility has led to the development of several indazole-based drugs. These properties make indazole a valuable structure in the development of therapeutic agents, highlighting its significance in pharmaceutical research. [4]

1.2 Microwave assisted synthesis:

Microwave heating has become a highly effective method for driving various chemical reactions. When used in solvent-free conditions, microwave reactions offer advantages such as reduced environmental impact, cost-effectiveness, and simplicity in both processing and handling. The recent advancement of single-mode technology ensures safer and more consistent experiments, contributing to the widespread adoption of microwave synthesis within the chemistry community. As a result, there has been a significant increase in publications on microwave-assisted synthesis, covering a wide range of chemical reactions, including additions, cycloadditions, substitutions, eliminations, and fragmentations. [5] [6]

Microwave irradiation has revolutionized organic synthesis over the past 30 years by reducing reaction times and increasing yields. It allows precise thermal control, minimizes chemical waste, and eliminates side reactions, making it an efficient, cost-effective method. Initially reported in 1969, microwave synthesis gained popularity due to the work of Gedye and Giguere. This method works through dielectric heating, where polar molecules rapidly absorb microwave energy, accelerating reactions compared to conventional heating. Microwave heating offers advantages such as shorter reaction times, higher yields, cleaner reactions, and fewer by-products, making it an eco-friendly and sustainable approach in chemical synthesis. [7]

1.3 Mechanism of microwaves:

Microwave heating works through dielectric heating, where the electromagnetic field generated by microwave radiation interacts with molecules, causing them to rotate and accelerating ions in the sample. This interaction leads to molecular friction, resulting in rapid temperature increase. Unlike traditional heating methods, which can be uneven, microwave heating produces more uniform heat. The radiation causes molecules with a dipole moment to align with the electromagnetic field, generating localized "superheating" as the molecules return to a disordered state. This process is highly efficient and homogeneous, making it effective for heating. [8]

2. Objectives

The objective of this study is to synthesize a series of novel indazole derivatives and evaluate their pharmacological activities. Specifically, the main objective is to **Synthesize** a range of indazole derivatives through optimized synthetic routes.**Characterize** the chemical structures of the synthesized compounds using techniques like NMR, IR, and mass spectrometry.**Evaluate** the pharmacological activities of these indazole derivatives, focusing on their potential as therapeutic agents for conditions such as fungal infection. **Correlate** the chemical structure with pharmacological activity to identify structure-activity relationships (SAR).

3. Methods

Raw material characterization:

Table 1: Raw material characterization					
Sr. no.	**Name of Chemicals**	**Molecular formula**	**Molecular weight**	**Melting point**	**Boiling point**
1	o-chloro benzaldehyde	C7H5ClO	140.57	10°C	212°C
2	o-nitro benzaldehyde	C7H5NO3	151.12	45°C	153°C
3	2,6-Dichloro benzaldehyde	C7H4Cl2O	175.01	71°C	231°C
4	Hydrazine hydrochloride	N2H4HCl	68.51	169°C	213°C
5	Distilled water	H2O	18.015	0°C	100°C

Experimental scheme:

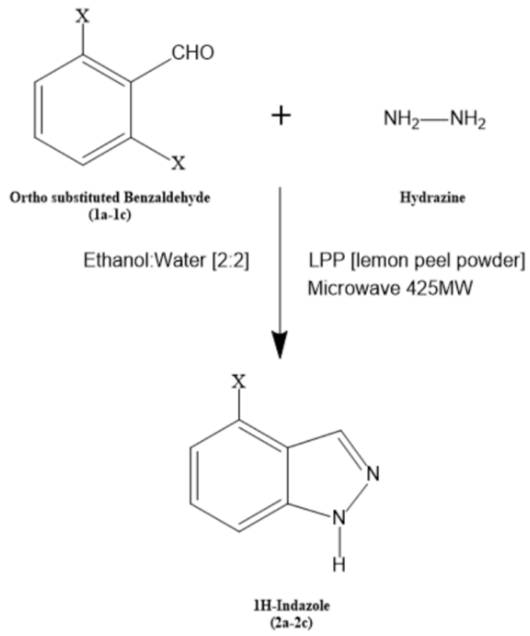


Figure 1: General scheme

Table 2: Reagent & Product		
Sr.no.	**Reagent**	**Product**
1	o-chloro benzaldehyde	1-H indazole
2	o-nitro benzaldehyde	1-H indazole
3	2,6-Dichloro benzaldehyde	4-chloro-1-H

Procedure for synthesis:

1. **Synthesis of 1-H indazole (derivative 2a):** A solution of o-chloro benzaldehyde (1 mmol), hydrazine hydrate (2 mmol), and LPP (10%) in 10mL of distilled water was treated with ultrasonic irradiation for 18min at 425MW. Reaction progress was monitored by TLC (n-hexane: ethyl acetate, 5:5). After completion, the mixture was diluted with hot ethanol and filtered. The catalyst was washed with ethanol (3 × 5 mL), and the combined filtrates were concentrated to obtain the crude product. This was then purified by recrystallization in ethanol. [9]

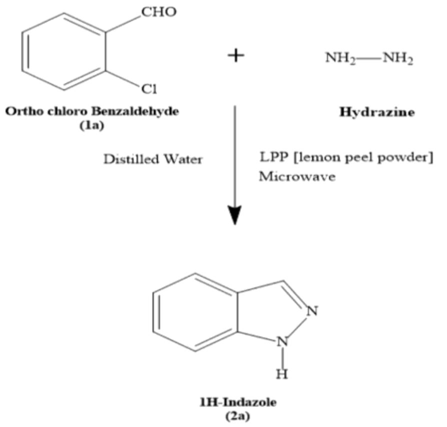


Figure 2: Synthesis scheme of 2a

2. **Synthesis of 1-H indazole (derivative 2b):** A solution of o-nitro benzaldehyde (1 mmol), hydrazine hydrate (2 mmol), and LPP (10%) in 10mL of distilled water was treated with ultrasonic irradiation for 18min at 425MW. Reaction progress was monitored by TLC (n-hexane: ethyl acetate, 5:5). After completion, the mixture was diluted with hot ethanol and filtered. The catalyst was washed with ethanol (3 × 5 mL), and the combined filtrates were concentrated to obtain the crude product. This was then purified by recrystallization in ethanol. [9]

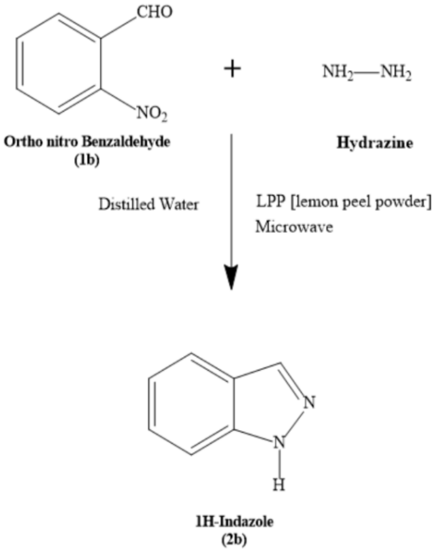


Figure 3: Synthesis scheme of 2b

3. **Synthesis of 4-chloro-1-H indazole (derivative 2c):** A solution of 2,6-dichloro benzaldehyde (1 mmol), hydrazine hydrate (2 mmol), and LPP (10%) in 10mL of distilled water was treated with ultrasonic irradiation for 18min at 425MW. Reaction progress was monitored by TLC (n-hexane: ethyl acetate, 5:5). After completion, the mixture was diluted with hot ethanol and filtered. The catalyst was washed with ethanol (3 × 5 mL), and the combined filtrates were concentrated to obtain the crude product. This was then purified by recrystallization in ethanol. [9]

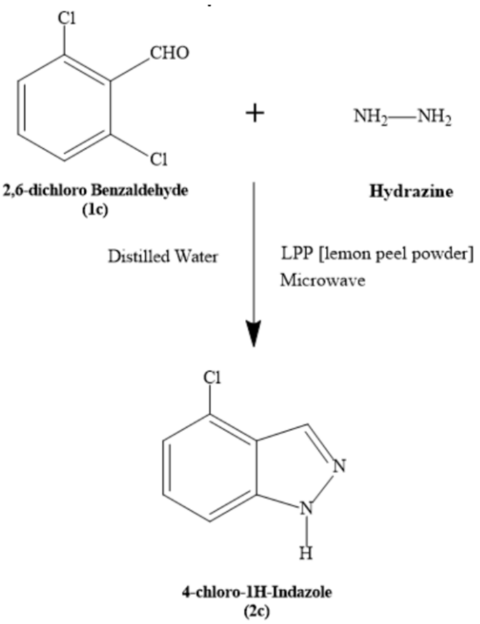


Figure 4: Synthesis scheme of 2c

4. Results

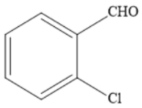
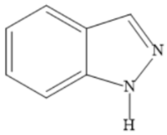
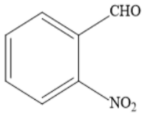
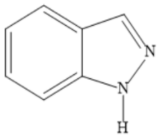
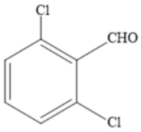
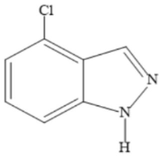
Identification and characterization: The identification and characterization of the compounds was carried out by the following procedures to ascertain that all prepared compounds were of different chemical nature than the respective parent compound**.**

1. Melting point determination: The melting point of the organic compounds were determined by open capillary tube method. Melting point is a valuable criterion of purity for an organic compound as a pure crystal is having definite and sharp melting point. The purity should not

be assumed but must be established by observation of any changes in the melting point. When the compound is subjected to purification by recrystallization. The synthesized compounds showed a minute change in a melting point after recrystallization.

2. Thin Layer Chromatography: TLC was performed on pre-coated silica gel plates using n-hexane and ethyl acetate (5:5, v/v) as the mobile phase. A small amount of the sample was applied to the plate using a capillary tube, ensuring the application was made at a uniform distance from the baseline. The plate was then placed in a developing chamber containing the mobile phase, and the solvent was allowed to ascend the plate by capillary action. Once the solvent front reached approximately 3/4 of the plate's length, the plate was removed and allowed to air dry. The separated components were visualized under UV light, and the Rf values were calculated by measuring the distance travelled by each spot relative to the solvent front. [10]

Table 3: Melting point, % yield and Rf value

Sr.no.	**Aldehyde**	**Product**	**Reaction time**	**% yield**	**Melting point**	**Rf value**
1			15min	77%	145°C	0.76
2			15min	81.5%	145°C	0.69
3			15min	85.79%	153°C	0.589

3. Infrared Spectroscopy (IR Spectroscopy): Infrared (IR) spectroscopy is a fast, non-destructive technique used to analyse the chemical composition of various samples. The infrared spectrum is divided into three regions: near-infrared (NIR), mid-infrared (MIR), and far-infrared, with NIR being more commonly used in food analysis due to its lower cost and better sample penetration. NIR spectroscopy measures overtones and combination bands of IR-active bonds, providing quick results without extensive sample preparation. It offers advantages such as sensitivity, minimal sample requirements, and the ability to analyse solids, liquids, and gases, making it a versatile tool in analytical chemistry. [11]

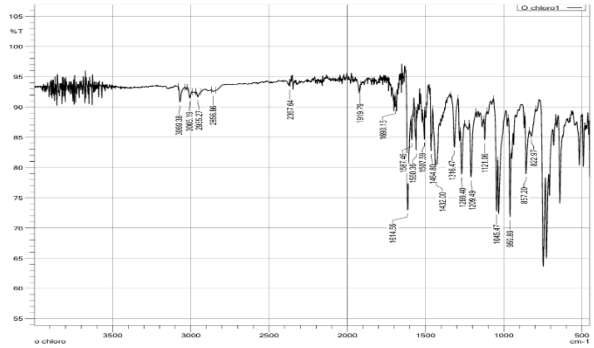


Figure 5: IR of 2a

Table 4: IR stretching of 2a			
Sr.no.	**Frequency**		**Functional group**
	Range	**Reported**	
1	1400-1600	1432-1587.46	C-C
2	1600-1700	1614.56-1690.15	C=C
3	3000-3100	3005.19-3069.38	C-H
4	1600-1650	1614.56	C=N
5	1300-1350	1316.47	N-N
6	3300-3500	3069.38	N-H

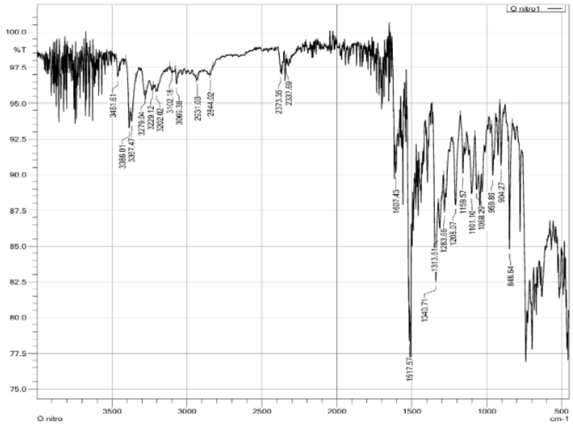


Figure 6: IR of 2b

Table 5: IR stretching of 2b			
Sr.no.	**Frequency**		**Functional group**
	Range	**Reported**	
1	1400-1600	1517.57	C-C
2	1600-1700	1607.43	C=C
3	3000-3100	3069.38-3102.18	C-H
4	1600-1650	1607.43	C=N
5	1300-1350	1313.61-1340.71	N-N
6	3300-3500	3367.47-3461.61	N-H

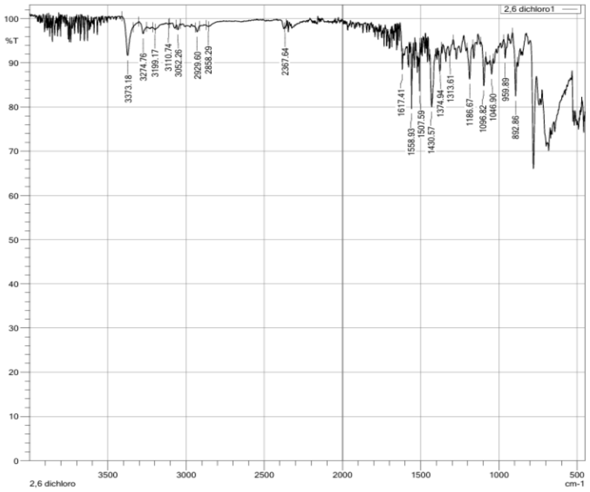


Figure 7: IR of 2c

Table 6: IR stretching of 2c			
Sr.no.	**Frequency**		**Functional group**
	Range	**Reported**	
1	1400-1600	1430.57-1558.93	C-C
2	1600-1700	1617.41	C=C
3	3000-3100	3052.26-3110.74	C-H
4	1600-1650	1617.41	C=N
5	1300-1350	1313.61	N-N
6	3300-3500	3373.18	N-H
7	600-800	892.86	C-Cl

4. 1H NMR Spectroscopy: 1H NMR (Proton Nuclear Magnetic Resonance) is a method used to study the hydrogen atoms in a molecule, offering insights into their bonding and surroundings. It’s a valuable tool for determining the structure of organic compounds. The technique is known for its high sensitivity, low sample requirement, and its ability to provide detailed structural information without altering the sample. [12] [13]

2a (1-H Indazole): 1H NMR (500 MHz, cdcl3) δ 9.08 (s, 1H), 8.21 (dd, J = 7.7, 1.2 Hz, 1H), 7.47 – 7.30 (m, 3H).

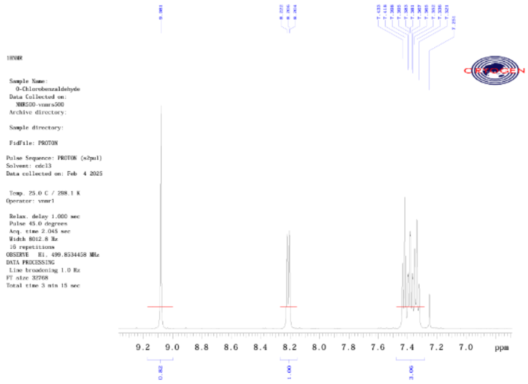


Figure 8: NMR of 2a

2b (1-H Indazole): 1H NMR (500 MHz, cdcl3) δ 8.25 (s, 2H), 8.04 (d, J = 7.7 Hz, 2H), 7.94 (t, J = 9.6 Hz, 2H), 7.60 – 7.53 (m, 2H), 7.44 – 7.37 (m, 2H), 5.95 (d, J = 38.8 Hz, 5H).

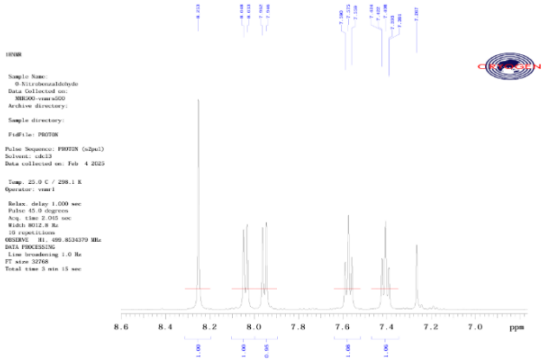


Figure 9: NMR of 2b

2c (4-chloro-1-H Indazole): 1H NMR (500 MHz, cdcl3) δ 7.92 (s, 2H), 7.32 (d, J = 8.1 Hz, 4H), 7.14 (t, J = 8.1 Hz, 2H), 5.99 – 5.71 (m, 5H).

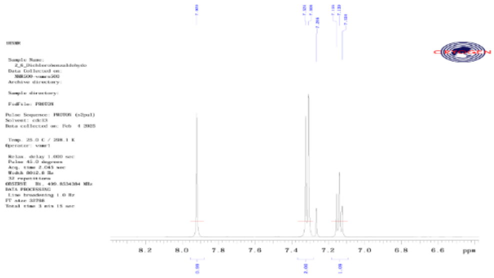


Figure 10: NMR of 2c

Anti-fungal activity:

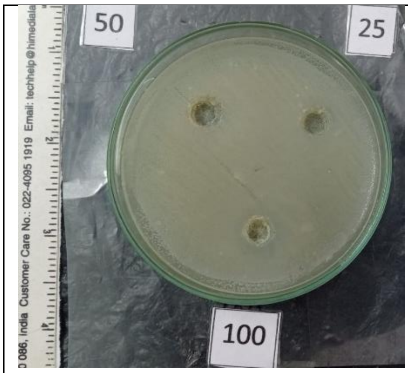
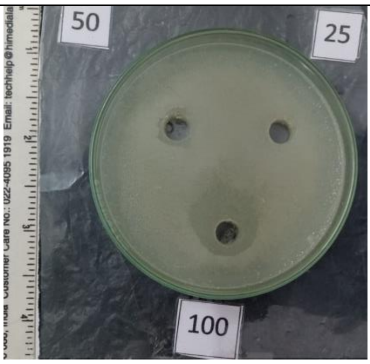
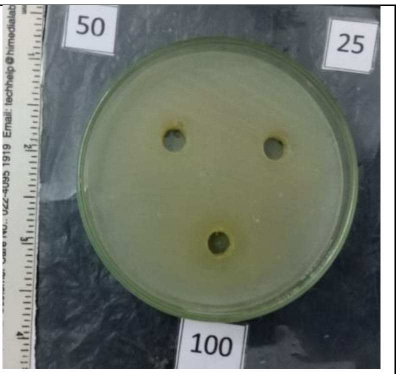
Methodology:

The Antifungal activity of 1-H indazole (2a), 1-H indazole (2b), 4-chloro-1-H indazole (2c) was performed by Kirby-Baur well diffusion method (Bauer et.al.,1966). 24 hours freshly grown (0.5 McFarland turbidity Standard) cultures of Strain CA13-*Candida albicans* in Potato dextrose broth medium were inoculated by lawn culture technique on sterile solidified Potato Dextrose Agar (Himedia – MH096) plates with the help of sterile cotton swabs.

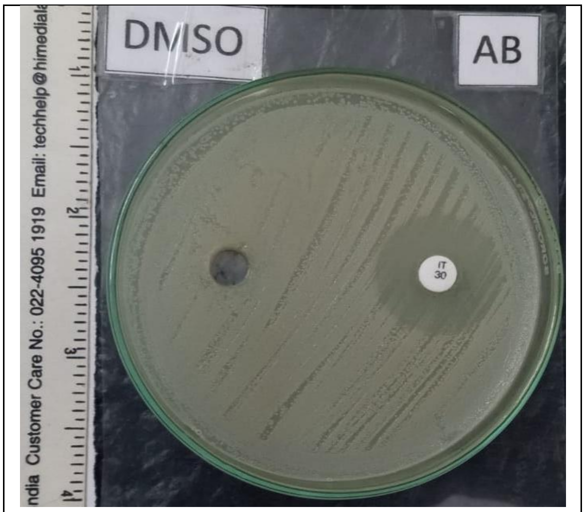
The stock solutions of concentration 1mg/ml were prepared in DMSO solvent. Three wells were bored on the MHA media plates with the help of sterile corkborer (8mm). Each well loaded with solution following concentration 25µl(25µg), 50µl(50µg) ,100µl(100µg) respectively. Then allowed to diffuse at room temperature for 2-3 hours. The plates were incubated in the upright position at 30 °C for 24 hours. Then the zone of inhibition was observed. [14] [15] [16]

The control plates were kept in which DMSO 100 µl was added as negative control and Antibiotic disc as a positive control were used. For *Candida albicans* Itraconazole 30 mcg were used. [17]

Table 7: Antifungal activity			
Zone Of Inhibition in mm			
Organisms	**25µl**	**50µl**	**100µl**
CA13- 1-H indazole (2a)	NI	NI	NI
CA13- 4-chloro-1-H indazole (2c)	NI	NI	17
CA13- 1-H indazole (2b)	NI	NI	NI
Note:-NI – No Inhibition ,Strain no CA13- *Candida albicans*			

		
CA13- 1-H indazole (2a)	**CA13- 4-chloro-1-H indazole (2c)**	**CA13- 1-H indazole (2b)**

Control	**Itraconazole 30 mcg (AB)**	**DMSO**
Candida albicans	20	NI
Note: -NI – No Inhibition, AB-Antibiotic		



Candida albicans

From the above results, it was observed that from all the three derivatives 4-chloro-1H indazole shows anti-fungal activity at 100µl. Standard drug Itraconazole shows anti-fungal activity at 30µl.

5. Discussion

A straightforward and highly effective microwave-assisted synthesis approach has been developed. This method allows for the successful production of indazole derivatives with high yields and reduced reaction times. It has been shown to be environmentally friendly, making it a practical option for creating new indazole derivatives for potential antifungal applications.

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