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SYNTHESIS & BIOLOGICAL EVALUATION OF PYRAZOLINE DERIVATIVE AS ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY

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Abstract: The pyrazoline derivatives as antioxidant and antimicrobial agents were synthesized. The pyrazoline derivatives were prepared in two steps firstlt the chalcones were prepared then converted in to final pyrazoline derivatives in presence of semicarbazide and methanol. The %Yield was calculated. Pyrazolines were characterized by molecular formula, molecular weight, physical state, color, melting point, solubility and R_f -Value. Also IUPAC name and Elemental Analysis was done. Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). *In-Vitro* tests are used as screening procedure for new agents and for testing susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent.

Keywords: Pyrazoline, Antioxidant, Synthesis, Semicarbazide, Synthesis, Antimicrobial

INTRODUTION

The pervasiveness of this heterocycle leads to multiple useful properties and has stimulated the need for facile and efficient ways to make these heterocyclic cores[1]. Moreover, the incorporation of the pyrazoline moiety and other electron-donors/ acceptors into one molecule is one of the most actively pursued areas of research involving fascinating diverse heterocycles, and seems to be a good choice for many applications[2]. The incorporation of different substituents into the pyrazoline parent nucleus is quite interesting, since it increases the conjugation length, due to the presence of an additional double bond in alternate arrangements with the phenyl ring, prompting researchers to synthesize and elucidate the effects of substituent on the absorption and fluorescence properties of this class of compounds[3].

Compared to the unsubstituted parent molecule, the incorporation of electron-donors and electron-acceptors into one molecule leads to endowment with structural diversity, stability and highly improved optical properties[4]. Of the factors influencing the nonlinear optical features of push–pull type pyrazoline based chromophores, pride of place must go to the position as well as the number and type of the electron-acceptor groups attached to the molecular structure[5].

In the last decade, great attention has been paid on the pyrazoline derivatives due to their unique molecular structure with simplicity of preparation and wide application in pharmaceutical field[6]. They have shown interesting pharmacological activities such as antimalerial, anticancer, anti-inflammatory, antimicrobial, antioxidant and antidepressant[7].

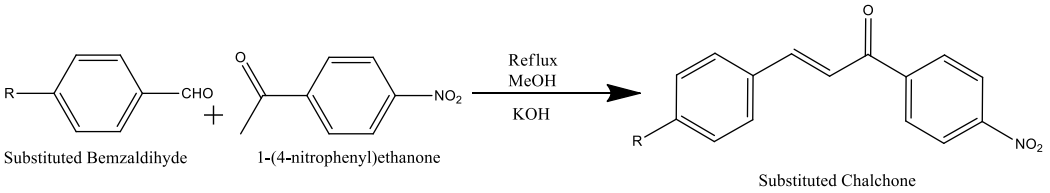
In continuation of the development of pyrazolines as promising candidate for drugs, a comprehensive data result regarding their status in global research community is urgently required[8]. So the main objective of the study is to synthesize the pyrazoline derivatives as antioxidant and antimicrobial agents.

MATERIALS AND METHOD

General method of synthesis of pyrazoline derivatives:

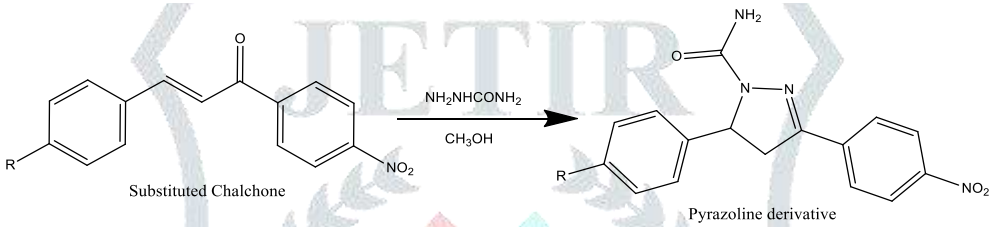
(A) STEP-I: General procedure for the preparation of chalcones: Equiv. molar mixture of 1-(4-nitrophenyl)ethanone (0.01 mol) and substituted benzaldihyde (0.01 mol) was stirred in ethanol (30 ml) and then an aqueous solution of KOH (40%, 15 ml) added to it.

The mixture was kept overnight at room temperature and then it was poured into crushed ice and acidified with HCl. Separated solid was filtered and crystallized from ethanol.



Substituted Bemzaldihyde 1-(4-nitrophenyl)ethanone Substituted Chalchone

(B) STEP-II: Procedure for the preparation of pyrazoline derivatives: The pyrazoline derivatives were synthesized by condensation of chalcone with semicarbazide in the presence of methanol (CH_3OH) and KOH. Completion of reaction was confirmed by TLC. Final obtained crude products were crystallized from ethanol. Using this method, products were synthesized with moderate yields.



Substituted Chalchone Pyrazoline derivative

Screening of Biological Activity of Synthesized Compounds

(A) Antioxidant activity by DPPH Assay method: Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 1.0 ml of sample solution with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/ml) was added to a 4 ml of 0.004% methanolic solution of DPPH. The absorbance was read at 517 nm after 30 min incubation at room temperature in the dark. Ascorbic acid was used as a standard. The DPPH radical-scavenging activity was calculated according to the following equation:

$$\textbf{DPPH scavenging activity } (\%) = \mathbf{1} - \frac{\mathbf{Ai - Aj}}{\mathbf{Ac}} \mathbf{X\ 100}$$

Where, Ac was the absorbance of DPPH solution without sample (2 ml DPPH + 2 ml of 95% methanol); Ai was the absorbance of the test sample mixed with DPPH solution (2 ml sample + 2ml DPPH) and Aj was the absorbance of the sample without DPPH solution (2 ml sample + 2 ml of 95% ethanol).

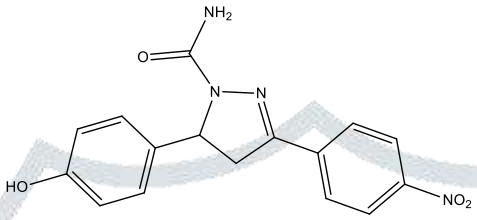
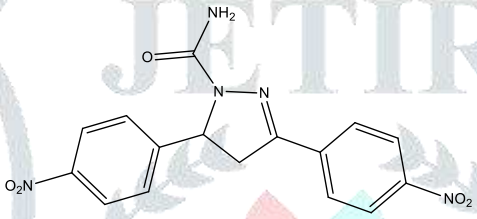
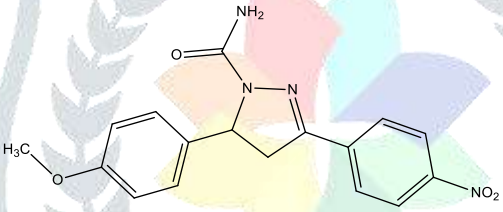
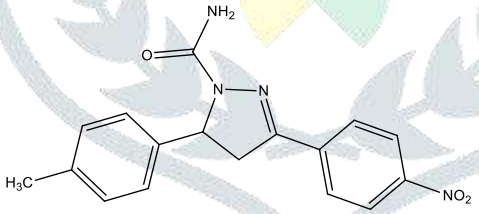
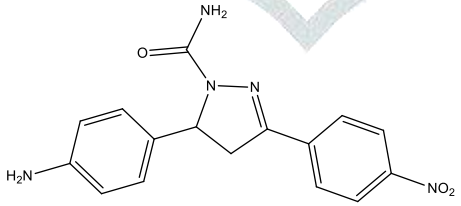
(B) *In-vitro* anti-microbial activity by Disc diffusion method: *In-Vitro* tests are used as screening procedure for new agents and for testing susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent. MIC is the smallest concentration of the substance required to inhibit the growth of a test organism under specified conditions.

(C) General procedure: Each petri plate containing nutrient/ sabouraud's agar medium was inoculated with one bacterial/ fungal culture by spreading the suspension of the organism with a sterile cotton swap. Each plate was divided into six equal portions along the diameter. Each portion was used to place one disk. Four disks of each sample were placed on four portions, one disk with standard drug and a disk impregnated with the solvent (DMF). All the plates were kept in the refrigerator for 30 minutes to allow the diffusion of the sample in to the refrigerator for 30 minutes to allow the diffusion of the sample into the surrounding agar medium. Then the plates inoculated with bacterial cultures were incubated at 37 ºC for 18 h and those with incubated at 25 ºC for 48 h. Diameter of the zones of inhibition wherever produced were measured and the average diameter for each sample was calculated. The diameters obtained for the test samples were compared with that produced by the standard antibiotic ciprofloxacin for antibacterial activity and griseofulvin and for antifungal activity

RESULTS AND DISCUSSION

Methods of Synthesis of Pyrazoline Derivatives

Table No. 3: List of synthesized compounds

Code	R	Structure	IUPAC Name
NP-1	-OH		5-(4-hydroxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
NP-2	-NO_2		3,5-bis(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
NP-3	-OCH_3		5-(4-methoxyphenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
NP-4	-CH_3		3-(4-nitrophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazole-1-carboxamide
NP-5	-NH_2		5-(4-aminophenyl)-3-(4-nitrophenyl)-4,5-dihydro-1H-pyrazole-1-carboxamide

Molecular and Physical Properties of Synthesized of Pyrazoline Derivatives

The molecular formula and molecular weight of synthesized pyrazoline derivatives were calculated and molecular weight was found as 324.33 to 355.30 g/mol., all were yellow to light yellow colored crystalline solids.

Table No. 4: Molecular and physical properties of synthesized compounds

S. No.	Compound	Molecular Formula	Molecular Weight	Physical state	Color
1	NP-1	$C_{16}H_{14}N_4O_4$	326.31	Crystalline solid	Light yellow
2	NP-2	$C_{16}H_{13}N_5O_5$	355.30	Crystalline solid	Yellow
3	NP-3	$C_{17}H_{16}N_4O_4$	340.33	Crystalline solid	Light yellow
4	NP-4	$C_{17}H_{16}N_4O_3$	324.33	Crystalline solid	Off-White
5	NP-5	$C_{16}H_{15}N_5O_3$	325.32	Crystalline solid	Yellow

Physical Properties of synthesized compounds:

The % yield: The % yield of synthesized pyrazoline derivatives were calculated and found as 54.27 to 67.61 % that was better % of yield for chemical compounds.

Melting point: The melting point of synthesis pyrazoline derivatives were obtained as 135-137 ^{O}C to 171-173 ^{O}C that was better for identified the individual chemical compounds.

Solubility: Most of the synthesized pyrazoline derivatives have greater solubility in Ethanol, Methanol, Chloroform and DMSO.

R_f value: The synthesized pyrazoline derivatives were run on the silica gel TLC plates and Rf-value of obtained as 5.1, 4.8, 4.3, 4.6, and 5.2 respectively.

Table No. 5: % Yield of synthesized compounds

S. No.	Compound Code	% Yield	Melting Point	Rf value
1	NP-1	58.92 %	135-137 ^{O}C	5.1
2	NP-2	56.75 %	152-153 ^{O}C	4.8
3	NP-3	54.27%	156-158 ^{O}C	4.3
4	NP-4	61.82%	147-149 ^{O}C	4.6
5	NP-5	67.61%	171-173 ^{O}C	5.2

Elemental analysis: Elemental analysis reveals the types and quantities of elements present in a sample, think of it as a chemical fingerprint.

Table No. 9: Elemental analysis of the synthesized compounds

Code	Molecular Formula	Calculated (found) % of Element			
		C	H	N	O
NP-1	$C_{16}H_{14}N_4O_4$	58.89 (58.78)	4.32 (4.28)	17.17 (17.11)	19.61 (19.52)
NP-2	$C_{16}H_{13}N_5O_5$	54.09 (54.02)	3.69 (3.61)	19.71 (19.62)	22.52 (22.62)
NP-3	$C_{17}H_{16}N_4O_4$	59.99 (59.76)	4.74 (4.67)	16.46 (16.39)	18.80 (18.62)
NP-4	$C_{17}H_{16}N_4O_3$	62.95 (62.82)	4.97 (4.86)	17.27 (17.18)	14.80 (14.71)
NP-5	$C_{16}H_{15}N_5O_3$	59.07 (59.01)	4.65 (4.53)	21.53 (21.41)	14.75 (14.62)

Spectral analysis of newly synthesized compound by FT-IR spectrograms:

IR spectra of synthesized derivatives showed characteristic peaks for the functional group present in the deivatives. The values of these vibrations are given in tables. All derivatives showed the fixed peak of N-H str.; N-O_2 str. and C-H str. (pyrazoline) with peak of substituted functional groups O-H str. (on NP-1), O-CH_3 str.(on NP-3), -CH_3 str.(on NP-4) and N-H_2 str.

The ^{1}H-NMR spectral analysis of synthesized compounds: The NMR (Nuclear Magnetic Resonance) spectrum of a synthesized pyrazoline compound**,** were revealed that the fixed δ- value present in each NMR were 8.33 (CH-Ar); 6.01(NH_2); 5.35 (OH); 4.91 (CH); and 3.94 & 3.69 (CH –Pyraz.) that were indicated the rigid and confirmed structure of pyrazolines. Some of the NMR peaks are changed according to substituted groups.

NMR spectra of NP-3:

Table No. 17: NMR interpretation of synthesized compound of NP-3

Peak No.	δ- value	Splitting	H	J- Value	Assigned Peak
1	8.33	d	2	7.25	CH-Ar
2	8.09	d	2	11.55	CH-Ar
3	7.18	d	2	8.95	CH-Ar
4	6.94	d	2	9.7	CH-Ar
5	6.01	s	2	-	NH2
6	4.91	T	1	2.65, 7.4	CH
7	3.94	m	1	-	CH –Pyrazo
8	3.83	s	3	-	O-CH3
9	3.69	m	1	-	CH –Pyrazo

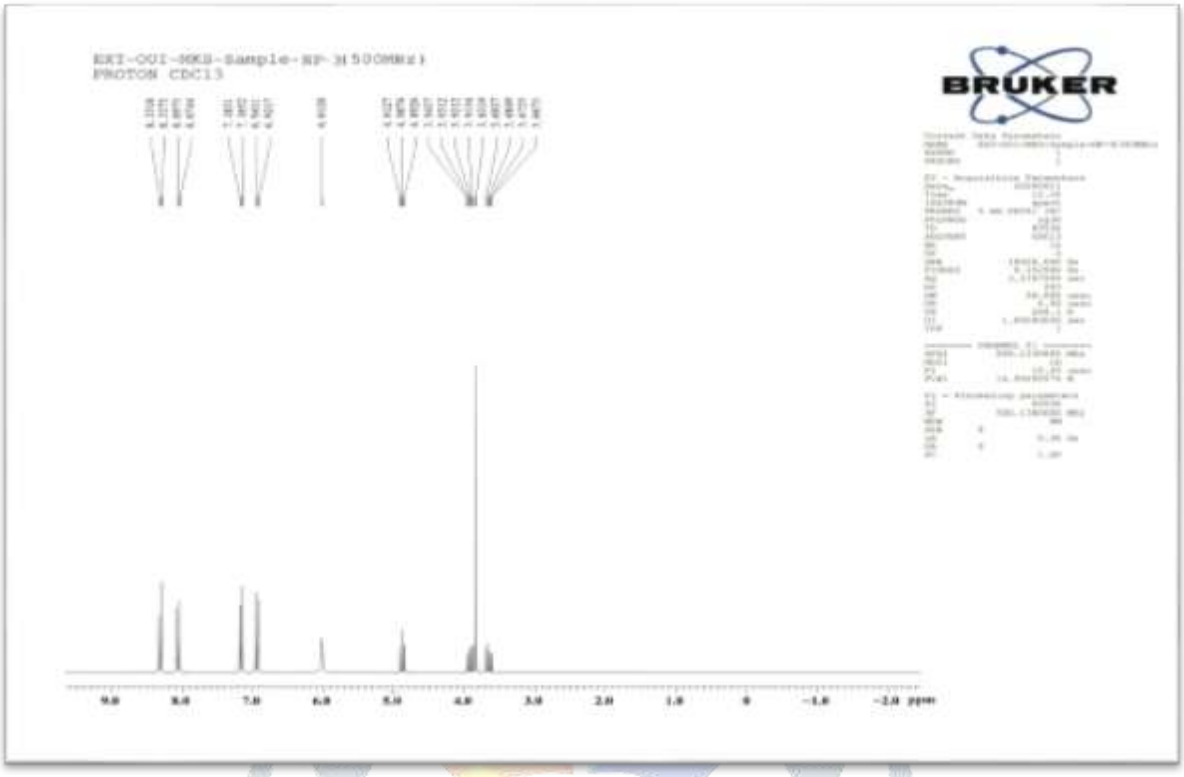


Figure 8: NMR interpretation of synthesized compound of NP-3

Screening of Biological Activity of Synthesized Compounds

(A) Antioxidant activity by DPPH Assay method: Free radical scavenging activity of samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH).

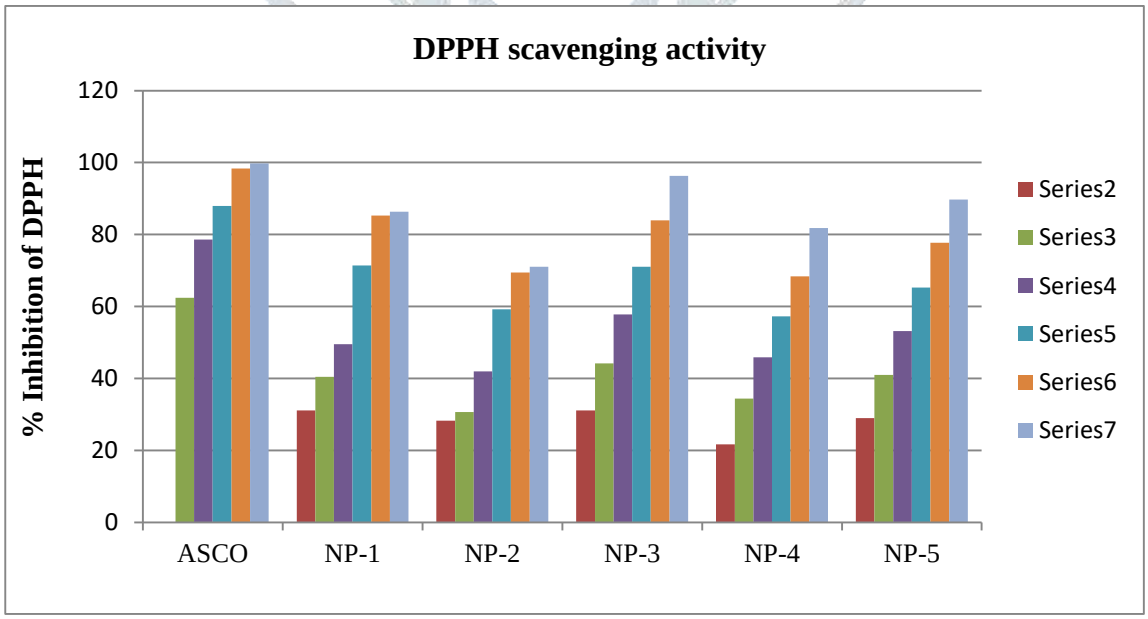


Figure 11: DPPH scavenging activity in the form of % inhibition

(B) *In-vitro* anti-microbial activity by Disc diffusion method: *In-Vitro* tests are used as screening procedure for new agents and for testing susceptibility of individual isolates from infections to determine which of the available drugs might be useful therapeutically. In general, minimum inhibitory concentration (MIC) and sensitivity tests are used to express the effectiveness of a compound as an antimicrobial agent.

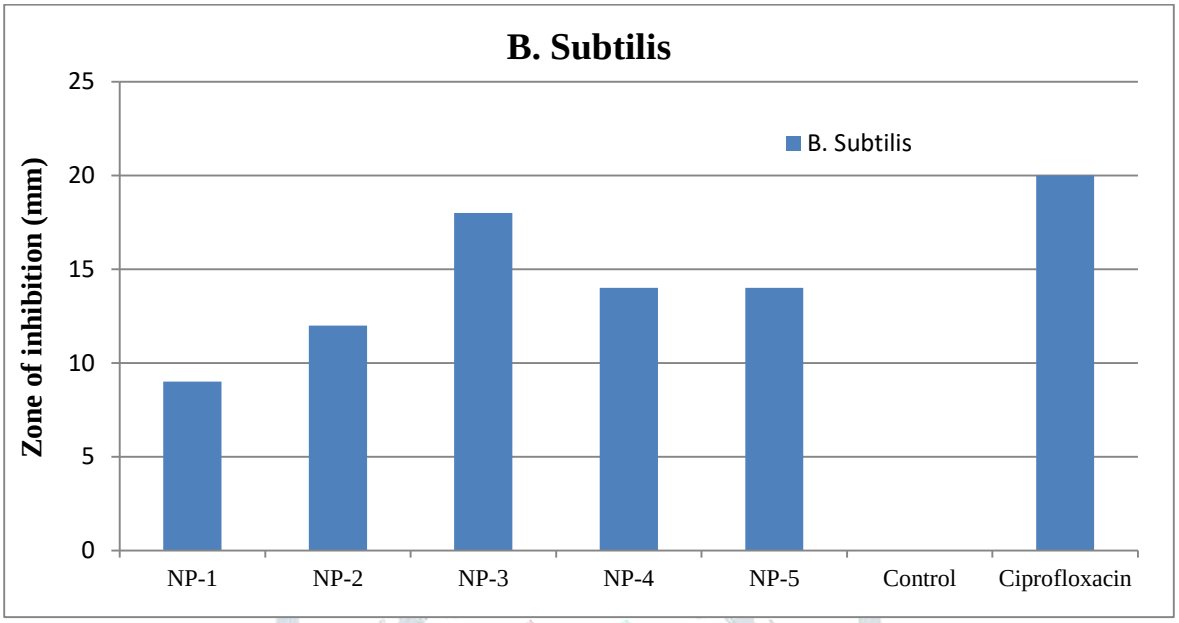


Figure 12: Anti Microbial Activity of synthesized compound against *B. subtilis*

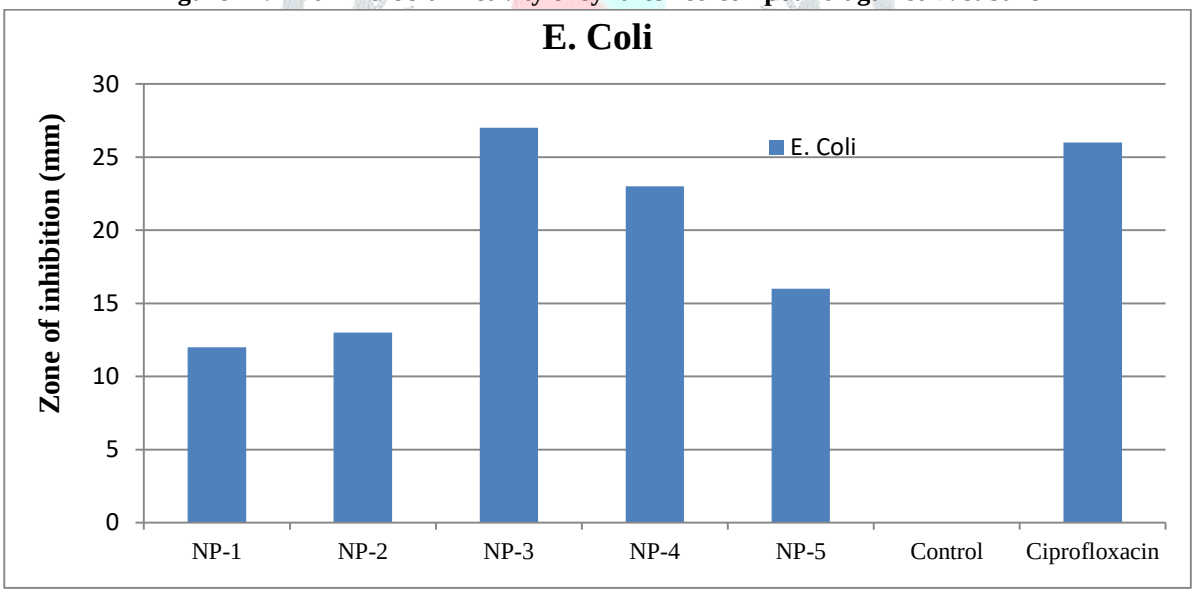


Figure 13: Anti Microbial Activity of synthesized compound against *E. coli*

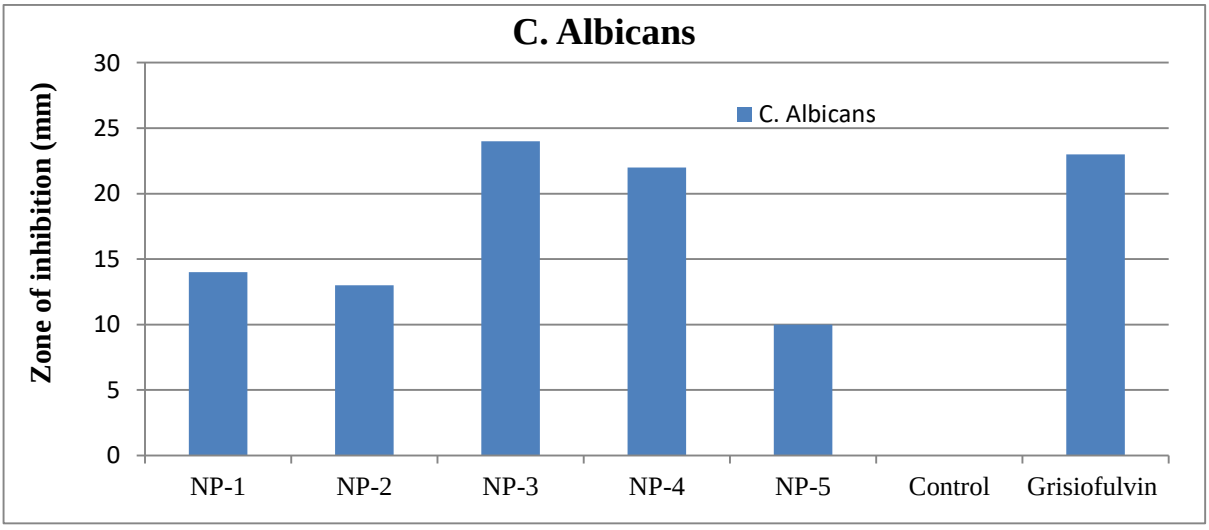


Figure 14: Anti Microbial Activity of synthesized compound against *C. albicans*

CONCLUSION

Pyrazoline derivatives are interesting groups of heterocyclic compounds exhibiting diverse pharmacological activities. They have wide range of applications starting from antimicrobial, anti-inflammatory, analgesic, antitubercular, anticancer, anti-HIV, antimicrobial to antidepressant and antihypertensive activities. Reported structure based drug design too gives an emphasis on Pyrazoline moiety. We thought that these models as such for synthesis give good opportunities to look for discovering ideal lead for antioxidant and anti-microbial activity. On the basis of this we had synthesized some new Pyrazoline derivatives with combination their substituted groups and then carried out test for their antioxidant and anti-microbial action. Further design may prove an alternative and very useful and fruitful in the discovery of new antioxidant and anti-microbial activity in comparison to grisiofulvin, ciprofloxacin

CONFLICTS OF INTERESTS

There are no conflicts of interests.

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