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Exploration of Spectral, Thermal, Antibacterial and Antioxidant Activities of newly synthesized inclusion complexes of N-(3-Phenylallylidine) and N-(3-isopropylbenzylidene)-(1, 3, 4) Thiadiazino[6, 5b]Indole-3-amine with β -CD

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ABSTRACT

In the context of the present medicinal advancement, the research has been carried out to synthesize and improvise some Indole derivatives having pharmacological activity through inclusion complex formation with β -cyclodextrin. The thermodynamic stability of the inclusion complexes are determined by studying different parameters such as free energy change and stability constant. The compounds and their inclusion complexes are characterized on the basis of physical and spectral analysis (UV, FT-IR, ¹HNMR). The biological screening was done against three bacterial strains i.e. E.Coli, S.aureus and P. Vulgaris in order to explore their vital antimicrobial activities. The antioxidant activities are also studied. Both the antibacterial and antioxidant activities are noticeably enhanced after encapsulation into the cavity of β -cyclodextrin.

Key words: Substituted indoles, β -Cyclodextrin, Inclusion complex, antibacterial activity, antioxidant activity.

INTRODUCTION

Cyclodextrins are proficient in forming inclusion complexes with a variety of pharmacologically and biologically active molecules [1]. Inclusion complex with cyclodextrins are extensively used to enhance solubility, stability and bio accessibility of drugs [2-3]. The inclusion complex formation ability of cyclodextrins is mainly due to their structural aspect i.e. hydrophobic internal cavity and hydrophilic external surface[4-5].

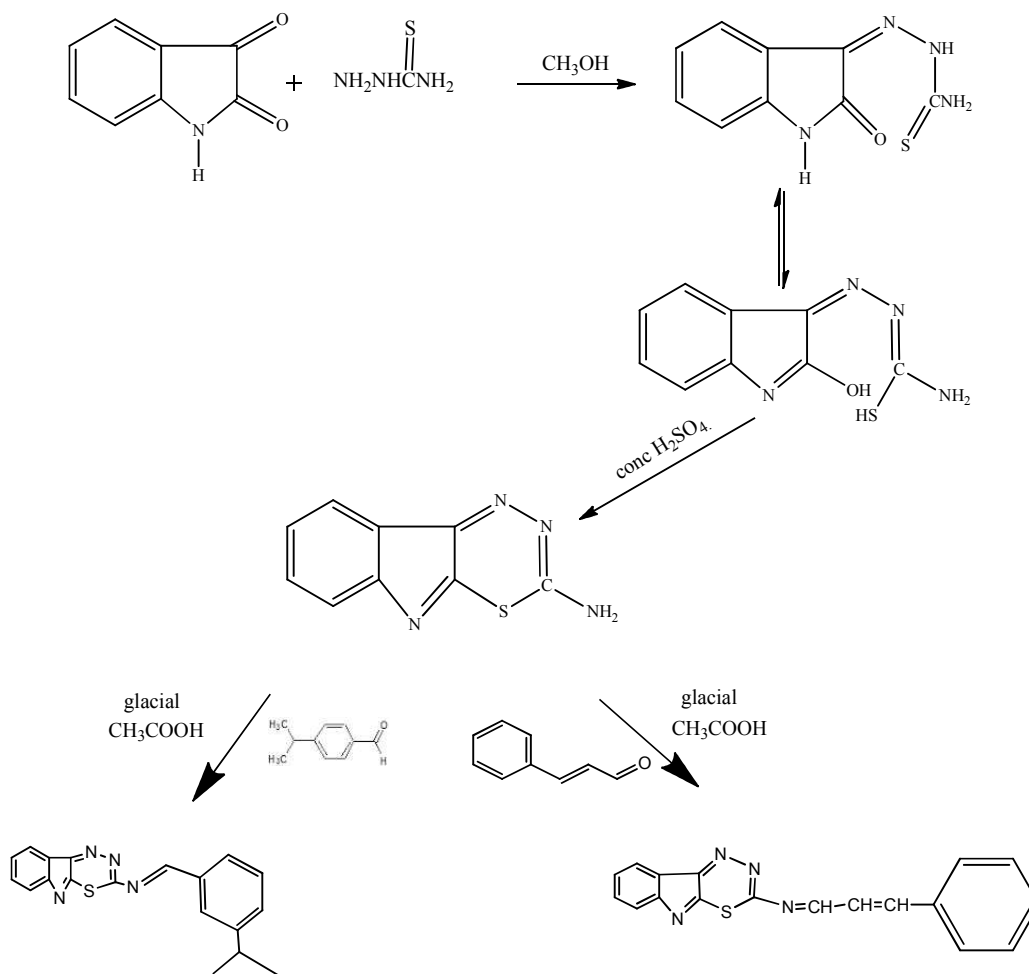
N-Heterocycles are widely known for their pharmacological potential and are associated with different bioactivities like antimicrobial, antioxidant, antitubercular, anticancer, antiHIV, antirheumatoidal etc[6-11]. Amongst N-Heterocycles, Indole and its analogous have diverse array of medicinal applications. In view of the above applications in medicinal chemistry, it appeared interests to synthesize N-(3-Phenylallylidine)-(1, 3, 4) Thiadiazino[6, 5b]Indole-3-amine and N-(3-isopropylbenzylidene)-(1, 3, 4) Thiadiazino[6, 5b]Indole-3-amine. The synthesized compounds are found to be sparingly soluble in polar solvent (water), which may be a restraining factor in decreasing their pharmacological activities. So with an objective of increasing the solubility and bio accessibility of these compounds, their inclusion complexes were prepared with β -cyclodextrin [12]. The formation and stability of inclusion complexes were ascertained by studying spectral(UV, IR, NMR), the thermodynamic stability constant, change in free energy etc.

Then the compounds and their inclusion complexes are screened against bacterial strains namely E.Coli, P.Vulgaris, Staphylococcus aureus etc. The compounds and their inclusion complexes are examined for antibacterial and

antioxidant activities in order to know their drug efficiency. The study reveals that the inclusion complexes are found to exhibit profound antibacterial and antioxidant activity as compared to their corresponding compounds, this may be due to increased solubility of the complex compounds.

MATERIALS AND METHODS

All chemicals and reagents used are of A.R grade (Sigma Aldrich). Double distilled water is prepared in the laboratory and is used throughout. Melting points are taken in open capillaries and are uncorrected. UV spectra is taken with the help of Shimadzu uv-1800 spectrophotometer and IR spectra are recorded on Shimadzu 8400 FTIR spectrophotometer using KBr pellets. ^1H NMR spectra in CDCl_3 are recorded on Bruker spectrophotometer model ultra-shield at 300 MHz using TMS as internal standard. Purity of the compounds is checked by TLC on silica gel plates.



Scheme-I

Synthesis of N-(3-Phenylallylidine) Thiadiazino [6, 5b] Indole-3-amine

N-(3-Phenylallylidine) Thiadiazino [6, 5b] Indole-3-amine is synthesized starting from indole-2,3-dione (as per the scheme-I) through the following intermediate steps.[13]

Step-1: Synthesis of 3-Thiosemicarbazido Indole-2-one

Methanolic solution of isatin and Thiosemicarbazide in equimolar ratio (0.013 moles) is mixed in a 500 ml Round bottomed flask. Refluxing is carried out for one hour. The completion of the reaction is checked by TLC. The contents are cooled and the excess of the solvent is removed. The formed compounds are collected after filtration

through Whatmann 42 filter paper. The residue is washed with distilled water. It is dried and recrystallized from methanol to obtain 3-Thiosemicarbazido Indole-2-one. Percentage of yield is 80% and the melting point is 235°C.

Step-2: Synthesis of 2-amino-1, 3, 4-Thiadiazino[6,5b] Indole

0.013 moles (3 grams) of 3-Thiosemicarbazido Indole-2-one is treated with small quantity of cold and concentrated H₂SO₄ in a beaker and the content is left at room temperature for 16 hours. Then ice cold water is poured in to the reaction mixture. Few drops of liquid ammonia are added to neutralise the mixture and in the process, a solid mass is obtained, which is filtered through Whatmann42 filter paper. The solid mass is washed with distilled water; it is dried and recrystallized from ethanol to give 2-amino-1, 3, 4-Thiadiazino [6,5b] Indole. The percentage of yield is 65% and melting point is 220°C.

Step-3: Synthesis of N-(3-Phenylallylidine) Thiadiazino [6, 5b] Indole-3-amine (Compound –A)

The equimolar mixture (0.01 moles) of Cinnamaldehyde and 2-amino-1, 3, 4-Thiadiazino [6,5b] Indole in 50 ml ethanol is refluxed for 6 hours in presence of glacial acetic acid(0.5ml) . The completion of the reaction is checked by TLC and excess of solvent is distilled out. The solution is cooled. To the clear solution crushed ice is added to get a solid product. It is filtered, washed with water and dried. The crude product is recrystallized from absolute alcohol to give the pure title compound.

By following the same procedure another compound i.e.N-(3-isopropyl benzylidene)-Thiadiazino [6,5b] indole-3-amine (**Compound-B**) is synthesized by taking equimolar mixture (0.01moles) of cuminaldehyde with 2-amino-1, 3, 4 -Thiadiazino [6,5b] Indole.

Phase Solubility Measurements :-

The aqueous phase solubility of the compound at various concentration. β -cyclodextrin (0-10mM) was studied by Higuchi-Corner method.[14]

Synthesis of inclusion complexes:

The inclusion complexes of the compounds (A and B) with β –cyclodextrin are prepared as per co-precipitation method [15-16]. The solutions of these compounds in required concentrations are added drop by drop to β –cyclodextrin solution of the required concentration. The mixtures are stirred for a period of 48 hours and filtered. The filtrate is cooled for 24 hours in refrigerators. The precipitate obtained is filtered through G-4 crucible, washed with water and dried in air for 24 hours.

Study of thermodynamic properties:-

The stability constants of the complexes (K) are calculated from plot of inverse of change in absorbance versus inverse concentration of β-cyclodextrin using Benesi-Hilderband relation[17].

$$1/\Delta A = 1/\Delta \epsilon + 1/K [Guest]_0 \Delta \epsilon [\beta\text{-CD}]$$

where ΔA is change in absorbance, Δε is change in absorption coefficient, K stability constant, [Guest]₀ is the concentration of compound and [β-CD] is the concentration of β-cyclodextrin. The values of K for all the complexes are calculated using the relation

$$K = \text{Intercept/Slope}$$

The value of ΔG at 298 K was calculated using the equation:

$$\Delta G = -RT \ln K$$

Antibacterial study

As per cup-plate method,[18] the antibacterial activities of the naked compounds and their inclusion complexes are undertaken against the bacterial species namely E. Coli, S.aureus and P. Vulgaris. The solutions of the test compounds were prepared in dimethylsulfoxide (DMSO) at 500µg/ml. The bacterial strains are inoculated into 100ml of the sterile nutrient broth and incubated at 37±1 °C for 24 hours. The density of the bacterial suspension is standardized by McFarland method. Well of uniform diameter (6mm) are made on agar plates, after inoculating them separately with the test organisms aseptically. The drug, control and the test compounds are introduced with the help of micropipette and the plates are placed in the refrigerator at 8-10°C for proper diffusion of drug into the

media. After two hours of cold incubation, the petriplates are transferred to incubator and maintained at $37 \pm 2^\circ\text{C}$ for 18-24 hours. Then the petriplates are observed for zone of inhibition by using vernier scale. The results are reported by comparing the zone of inhibition shown by the test compounds with standard drug Tetracycline. The results are the mean value of zone of inhibition of three sets measured in millimeter²⁵.

Evaluation of Antioxidant activity

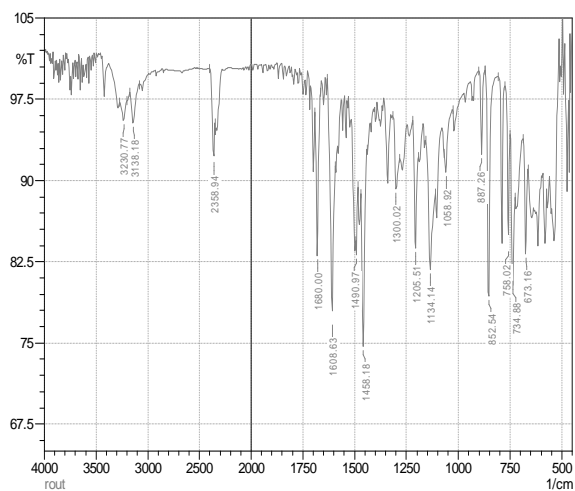
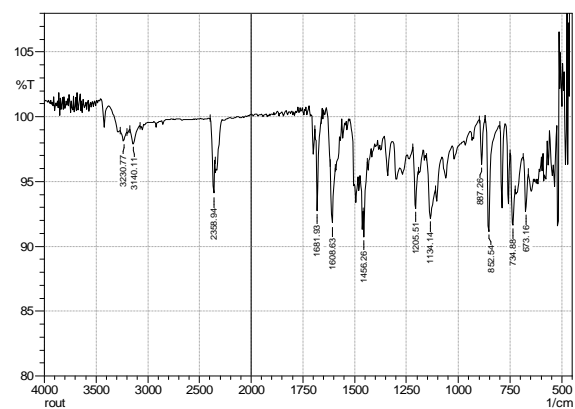
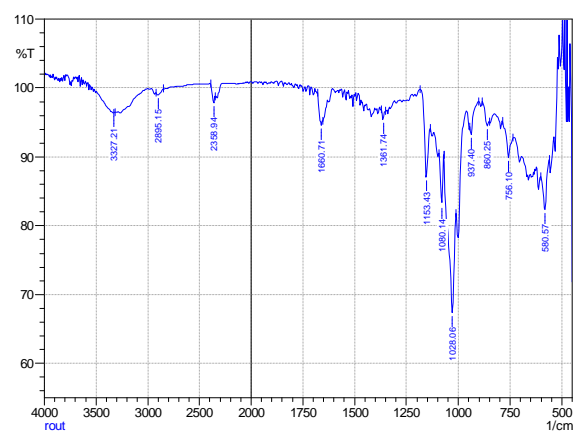
As per the method suggested by Tagashira and Ohtake [19], the antioxidant activity of the synthesized compounds was studied. DPPH (2, 2-Diphenyl-1-picrylhydrazyl) method is used for screening the compound. Ethanolic DPPH having $100\mu\text{g/ml}$ concentration is used to prepare sample solution for test. The mixture is incubated for 10 minutes at room temperature After vortexing with $100\mu\text{g/ml}$ concentration. The absorbances of the samples are calculated at 517 nm. The difference of absorbance between a test sample and a control is the activity of the sample. Here the reference substance used is Butylated Hydroxyl Toluene (BHT) (Table-4).

RESULTS AND DISCUSSION

The inclusion complexes of A and B have been prepared with β -cyclodextrin after determining the optimum concentration of host and guest through aqueous phase solubility study (Fig.5 and 6). From the study of their physical properties, IR and ^1H NMR data (Table-1) the structures of the compounds and their inclusion complexes are established. The synthesis of inclusion complexes of the compounds have been confirmed from the changes in melting point, colour and IR and ^1H NMR spectral characteristics (Table-1). From the melting point data it is revealed that inclusion complexes are having higher value than their corresponding compounds due to the fact that it requires an additional thermal energy to bring the compounds out of the cavity of β -cyclodextrin.

Table-1: Physical and spectral data of the compounds and their inclusion complexes

Sl.no	Compound	Colour	Melting point	Yield (%)	Molecular formula	M.W	IR (KBr) cm^{-1}	λ_{max} (nm)	NMR
1	A	Yellow	210 °C	70 %	$\text{C}_{18}\text{H}_{12}\text{N}_4\text{S}$	316	734 (C-S str.) 1490, 1485,1608 (Ar., C=C str.) 1134 (C-N str.) 1680(C=N str.) 3138 (C-H str.)	352	^1H NMR (CDCl_3): δ 7.30-7.80 (m, 5H, Ar-H), 7.40-7.60(d,4H, Ar-H),7.22-7.50(d,2H,C-H),5.67 (m,1H, C-H)
	I.C.A	yellowish-white	242 °C				756 (C-S str.) 1490, 1485,1608 (Ar., C=C str.) 1154 (C-N str.) 1680(C=N str.) 3157 (C-H str.) 3294-3327(-OH str. β -CD)	353	^1H NMR (CDCl_3): δ 7.11-7.8 (m, 5H, Ar-H), 7.20-7.40(d,4H, Ar-H),7.12-7.35(d,2H,C-H),5.39 (m,1H, C-H)
2	B	yellow	213 °C	76 %	$\text{C}_{19}\text{H}_{16}\text{N}_4\text{S}$	332	734(C-S str.) 1456,1608 (C-C str.)1134 (C-N str.) 1681(-C=Nstr.) 3140 (C-H str.)	350	^1H NMR (CDCl_3): δ 1.25 (d, 6H, CH_3), 7.25-7.85 (d,4H, Ar-H),2.84 (m,1H,C-H),8.25 (s,1H, C-H)7.63(s,1H, Ar-H),7.30-7.52(m,3H,Ar-H)
	I.C.B	Pale yellow	255 °C				752(C-S str.) 1475,1620 (C-C str.)1145 (C-N str.) 1696(-C=Nstr.) 3175 (C-H str.) 3254-3330(-OH str. β -CD)	352	^1H NMR (CDCl_3): δ 1.16 (d, 6H, CH_3), 7.11-7.66 (d,4H, Ar-H),2.68(m,1H,C-H),7.98 (s,1H, C-H)7.56(s,1H, Ar-H),7.25-7.43(m,3H,Ar-H)

**Fig.-1: IR spectra of N-(3-Phenylallylidene)1,3,4-Thiadiazino-[6,5b] indole-3-amine****Fig.-2: IR spectra of N-(3-isopropylbenzylidene)-1,3,4-Thiadiazino-[6,5b] indole-3-amine****Fig.-3: IR spectra of inclusion complex of N-(3-Phenylallylidene)1,3,4-Thiadiazino-[6,5b] indole-3-amine**

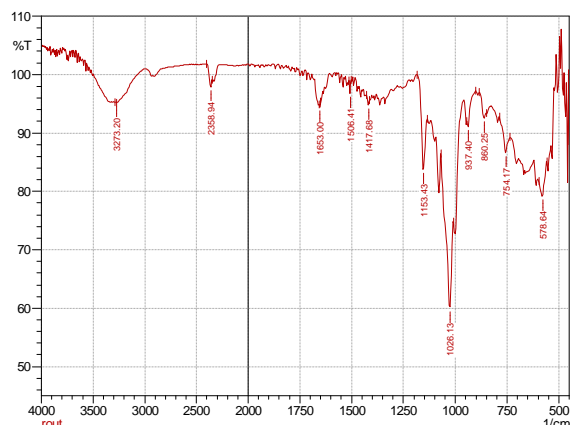


Fig.-4: IR spectra of inclusion complex of N-(3-isopropylbenzylidene)-1,3,4-Thiadiazino-[6,5b] indole-3-amine

The IR spectra of the compounds A and B and their inclusion complexes are shown in fig 1-4. The IR frequencies of the inclusion complexes show a noticeable change (broader and smoother) due to host guest interaction through the development of weak interactions like H-bonding, vander-Waal forces within the cavity of the host. [20,21] This observation clearly demonstrates transference of the compound from a hydrophilic environment to hydrophobic environment as the guest molecule is trapped in the host cavity (cavity of β -CD). In case of IR spectra of all the inclusion complexes a broad peak is obtained in the range $3250\text{--}3330\text{ cm}^{-1}$ due to the --OH stretching of β -CD. The encapsulation of the synthesized pharmacophore within the cavity of β -CD is further supported by ^1H NMR data (Table-2). The NMR data of the inclusion complexes show a little shift towards lower value of δ i.e. upfield. This shift in δ value confirms the formation of a new complex which may be due to the shielding mechanism in the cavity of the host. The aqueous phase-solubility diagrams of the compounds with β -cyclodextrin are shown in Fig. 5 and Fig. 6.

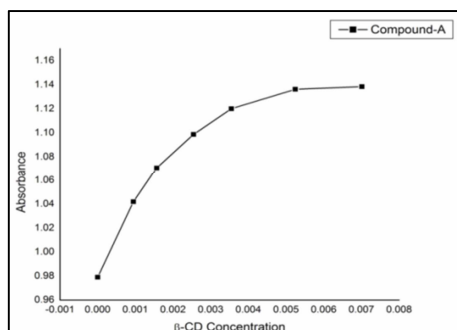
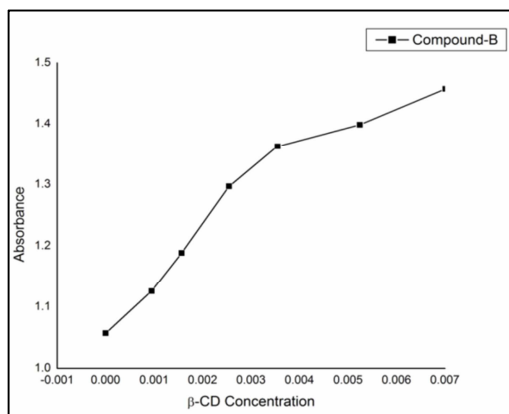
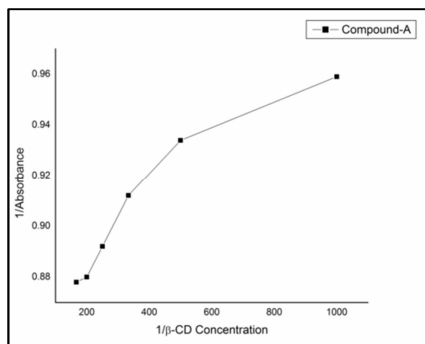
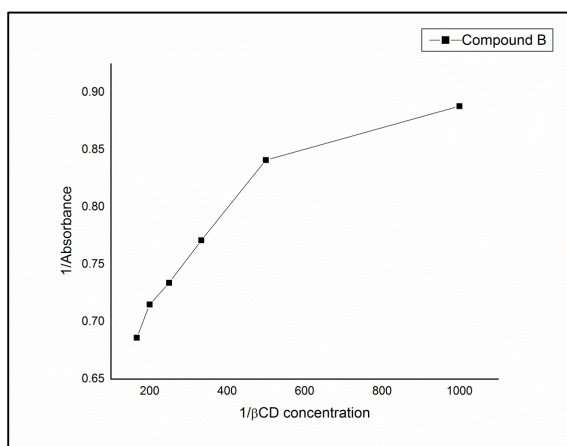


Fig.-5: Phase solubility of compound-A

**Fig.-6: Phase solubility of compound-B**

It is seen that aqueous solubility of the compounds increase linearly as a function of the concentration of β-cyclodextrin. The plot of inverse absorbance against inverse concentration of β-cyclodextrin gives straight lines with definite slope and intercept for different compounds (Fig. 7 and Fig.8).

**Fig.- 7: Plot of 1/ absorbance Vs 1/β- cyclodextrin conc. for compound A****Fig.- 8: Plot of 1/ absorbance Vs 1/β- cyclodextrin conc. for compound B**

The equilibrium constants (K) have been calculated for the inclusion complexes I.C._A and I.C._B from the slope and intercept and are found to be 318.3 and 568.6 respectively (Table-2) .

Table 2-Thermodynamic stability constant and free energy change of inclusion complexes

Sl No.	Inclusion complex of Compound	Equilibrium Constant K in M ⁻¹	$\Delta G = -2.303RT \log K$ ΔG (kJ/mol)
1	I.C.A	318.3	-14.27
2	I.C.B	568.6	-15.71

Since all the values are remaining within ideal range [22], complexes formed are quite stable. Further, it is found that the values of all the slopes are less than one indicating the inclusion complexes to have 1:1 stoichiometry [23]. Negative values of free energy changes for all the inclusion complexes (Table-3) further suggest that the process of inclusion complex formation is spontaneous and thermodynamically allowed. From the graphs of antibacterial studies against three bacterial strains namely *E. coli*, *S. aureus* and *P.vulgaris*, it is found that the diameter of the zone of inhibition of inclusion complexes noticeably high as compare to the compounds which shown in the fig. (Fig-9 and 10).

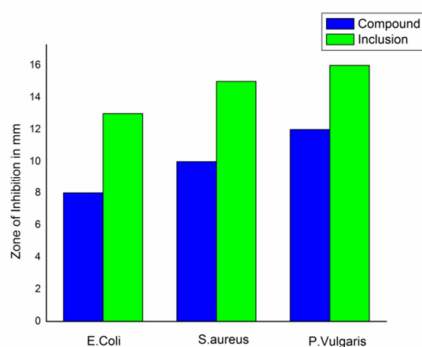


Fig.- 9: Zone of inhibition of compound A and its inclusion complex

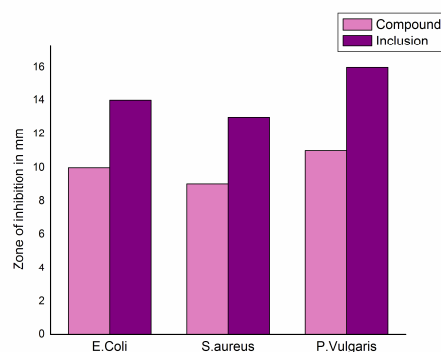


Fig.- 10: Zone of inhibition of compound B and its inclusion complex

The antibacterial activity of compound A shows very good result after encapsulation as compare to inclusion complex of compound B with respect to *S.aureus*. But for the other two bacteria (*E.Coli* and *P.Vulgaris*) the zone of inhibition is almost same. The remarkable enhancement of antibacterial activity of the inclusion complexes due to their solubility in the aqueous medium which makes them more bio-accessible and effective towards specific tissues thereby increasing drug efficiency.[24] After encapsulation, the radical scavenging activity of the compound increases significantly as shown in Table-3.

Table-3: Antioxidant activities of compounds and their inclusion complexes

Compound/Complex	Conc.(500 μ g/ml)	Conc.(100 μ g/ml)
Compound-A	36.6	28.9
Inclusion with β -CD	49.6	36.7
Compound-B	29.7	23.6
Inclusion with β -CD	45.4	32.8
Ascorbic acid	90.00	74.30

This can be associated with higher solubility of the compounds due to inclusion complex formation there by increasing the bio-accessibility. The free radical capturing capacity and interaction with reactive oxygen species of the compounds improved with the increase in bio-accessibility thereby increasing antioxidant activity of the compounds.[25]

CONCLUSION

An inclusion complex is formed between the synthesized compounds and β -cyclodextrin with the ratio 1:1. The synthesized pharmacophore and its inclusion are well characterized by the spectral data. The formation of inclusion complexes are thermodynamically allowed and having higher stability. The synthesized compounds are well fitted into the hydrophobic cavity of β -cyclodextrin which make them more soluble and bio-accessible. A noticeable increase in the antibacterial and antioxidant activities of the compounds have been observed after formation of inclusion complex.

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