Synthesis, Characterization, Enhanced Solubility and Antioxidant Activity of the Inclusion Compound of 2-Amino-N-o-tolyl-benzamide and Beta Cyclodextrin

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Summary: The objectives of this research, were to study the effect of beta cyclodextrin on the enhancement of the solubility and the antioxidant activity of 2-Amino-N-o-tolyl-benzamide (ATB). ATB was obtained by a new synthesis method and used to form inclusion compound macromolecule with β-CD. The characterization of the ATB and the complex were subsequently established by FT-IR; ^{1}H NMR; ^{13}C NMR; NMR 2D spectra and XRD. The optical activity was measured. The interaction of β-CD and ATB was also analyzed by UV-Vis spectroscopy in order to determine the formation constant. The stoichiometry of the complex was 1:1. Furthermore, the solubility and antioxidant activity of ATB were significantly enhanced upon complexation with β CD.

Key words: 2-Amino-N-o-tolyl-benzamide (ATB); inclusion compound antioxidant activity; reducing propriety.

Introduction

Benzamides derivatives were used as potential candidates for anti-alzheimer; antifatigue and antiurease [1]. Benzamides are an important group of compounds with a wide range of biological activities. Literature reviews have revealed that benzamides have medicinal applications, such as antiasthamatic [2]; anti-inflammatory [3] anthelmentic and antimicrobial. They reduce chronic myeloid leukemia; and also reduce tumor growth [4], 2-Amino-N-o-tolyl-benzamide (ATB) is obtained by sevral methods [5-6] and has an anticonvulsant activity against maximal electroshock [7]. Macromolecules were used to complex some drugs to increase their solubility [8] or to complex metals in order to change their electrical and magnetic properties [9]. In other cases for example they used the bochar whose structure is porous to incorporate macro and micronutrients with the aim of producting a slow release nanofertilizer for sustainable agriculture [10]. So in this work we will use the beta CD as a macromolecules, then, some guest molecules has been encapsulated in β-cyclodextrin in order to enhance their solubility and stability in aqueous solution. In aqueous solutions; CD is able to form inclusion compoundes [11-21] with a large variety of guest molecules; including organic compounds [6], pharmaceutical compounds [22] and organometallic compounds. [23]. The geometry of beta CD gives hydrophobic cavity having a depth of 7.8A˚; internal diameter of 6.5 A° and external diameter of 1.54 A° (Fig. 1a) [24]. In aqueous solution the slightly apolar CD cavity is occupied by water molecules that can be readily replaced by appropriate ‘‘guest molecules’’ which are less polar than water. Such guest molecules lead to the formation of an inclusion compound with CD; acting as host molecule. In the pharmaceutical industry; β -cyclodextrin can be used to reduce or prevent gastrointestinal and ocular irritation; reduce or eliminate unpleasant smells or tastes; prevent drug-drug or drug-additive interaction; or to convert oils and liquid drugs into microcrystalline or amorphous powders [25-26]. Herein, we report a study of the inclusion compound formed between 2-Amino-N-o-tolyl-benzamide (ATB)(Fig. 1b) and β-cyclodextrin (Fig. 1a); in aqueous medium. Analysis of our data by ^{1}H NMR and UVis data; confirm that the inclusion occurs and the complex has a 1:1 stoichiometry.

Experimental

Apparatus

FTIR spectra were recorded on Nicolet IR 200 FT-IR Thermo-Scientific spectro-photometer equipped with a diamond crystal. Spectra resolution was 4cm^{-1}. ^{1}H NMR experiments were carried out in a 300 spectrometer Bruker (^{1}H: 300 MHz; ^{13}C: 75.47 MHz). The chemical shifts (δ) are reported in ppm relative to TMS (internal reference). The ATB and supramolecular complex were dissolved in a mixture of $CDCl_3$ + DMSO-d_6 as solvent.

The powder X-ray diffraction patterns were obtained by a diffractometer PHILIPS PW 1729. The UV–Vis spectrum was recorded in the range 200–400 nm with a UV spectra photometer JENWAY 6405 equipped with a stoppered quartz cell with 1.0 cm optical pathlength.

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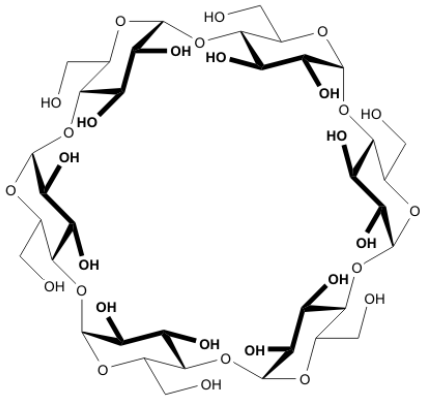
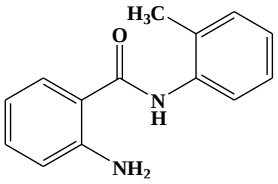


(1a)



(1b)

Fig. 1: a) β-cyclodextrin, b) 2-Amino-N-o-tolyl-benzamide (ATB).

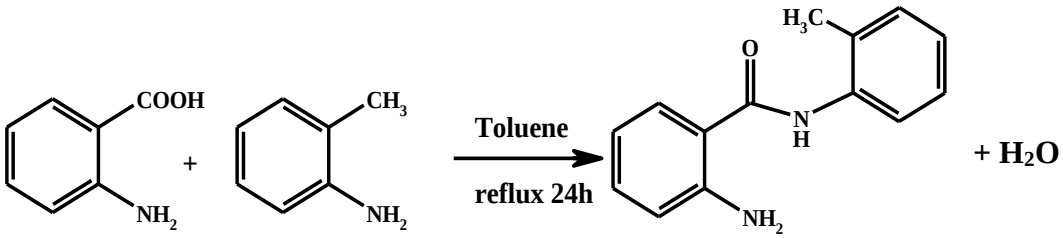
Chemicals and reagents

Anthranilic acid and o-tolylamine are commercial products. The other chemicals used were of analytical reagent grade without further purification.

Novel Method of Synthesis of ATB

The new one-step synthesis reaction of ATB is described in Scheme 1.

Ortho tolyl amin (0.6 mmol) and anthranilic acid (0.5 mmol) was dissolved in 20 mL toluene. The mixture was heated under reflux for 24 h. After evaporating the toluene from the reaction mixture the obtained precipitate was filtered and washed with ether. The ATB was recrystallized in ethanol. The ATB was obtained with a high yield (85%), white solid; MP: 104-106 °C. FTIR: ν_{C-H}: 3100- 3000 cm^{-1}; ν_{N-H} at 3500.05 cm^{-1}; $\nu_{C=O}$ = 1668 cm^{-1}; ν C–C_{arom} = 1650-1400 cm^{-1}. ^{1}H NMR (300 MHz; $CDCl_3$ + DMSO-d_6) d: 7.80; 7.24; 7.20; 6.90; 6.80; 6.65; 5.20; 2.40; ^{13}C NMR (75;47MHz; $CDCl_3$+ DMSO-d_6): 169.14; 150.80; 133.44; 131.15; 129.76; 126.29;125.6; 123.12 ;117.32; 116.03; 114.94; 114.38; 110.08; 16.83.



Scheme-1: Synthesis reaction of ATB.

Synthesis of inclusion compound

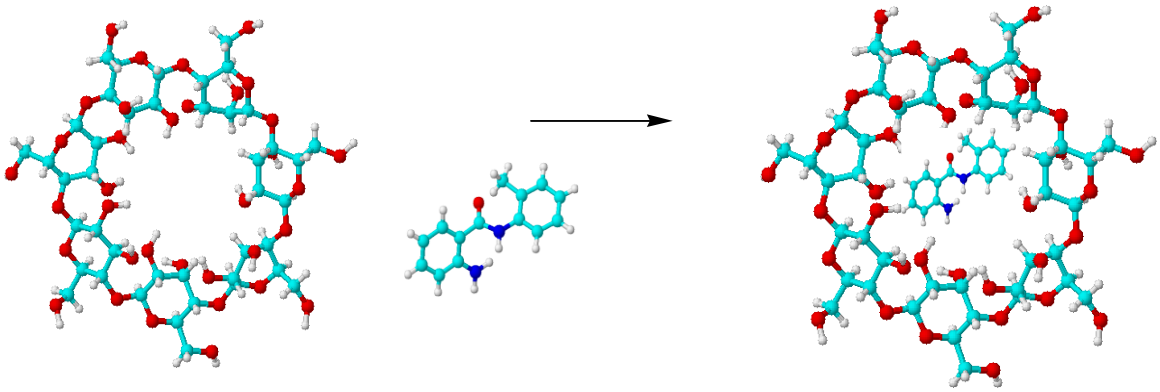


Fig. 2: Synthesis of inclusion compound of ATB and β-CD.

0.5×10^{-3}mol (0.113 g) of ATB dissolved in EtOH (10 mL) was dropped into 0.5×10^{-3}mol (0.5675 g) of β-cyclodextrin dissolved in 30 mL distilled water; with continuous stirring. The stirring operation was continued for 48 h at room temperature after which it gave a solid suspended products. The inclusion compound of ATB and β-CD was obtained by filtration. The precipitate was washed with ether four times, in turn; at room temperature for exact 24 h. (yield = 78%).

Results and Discussion

Our ATB was synthesized using one a pot method; with good yield, and characterized by X-ray powder diffractometry; UV analysis; IR and ^{1}H and ^{13}C NMR. The Inclusion compoundes of β-CD have mainly been used some times to increase the aqueous solubility of poorly water-soluble drugs; and to increase their bioavailability and stability. Our inclusion compound of β-CD and ATB was utilized to study the effect of the complexation on its solubility and antioxidant activity. The inclusion compound, which was prepared in 1:1 molar ratio by a co precipitation method [27-28]. We also studied its solubility and tested the antioxidant activity of the ATB and the complex.

Powder X-ray diffraction

The powder XRD patterns of the physical mixture in molar ratio 1:1 (guest:host); ATB and CD monomer were compared with the head of inclusion compound. The powder X-ray pattern of the inclusion compounds shown in Fig 3 was different from that of ATB and cyclodextrin free. The difference between the spectra of ATB; the cyclodextrin and the spectrum of the inclusion compound are due to the interactions of cyclodextrin with ATB, produces a new structure.

UV-visible analysis

The UV spectra shown in Fig 4, of (a) ATB, and the (b) physical mixture investigated have the same spectra and shows exact three absorption maxima. On the other hand, the UV spectrum of inclusion compound shows four absorption maxima. In the presence of beta-cyclodextrin the absorption at 336 ; 245 nm and 217nm were shifted to longer wavelengths **(**3-5 nm) along with some lowering of the absorption intensity. Concentrations of the sample to be measured were adjusted. Base line was established for each measurement; by placing in the reference compartment an aqueous solution for cyclodextrin; ethanol for ATB and a mixture of water and ethanol (80/20; v/v) for a physical mixture and inclusion compound at the same concentration (C $=10^{-5}$M). All measurements were carried out at 25.0 ± 0.01 °C.

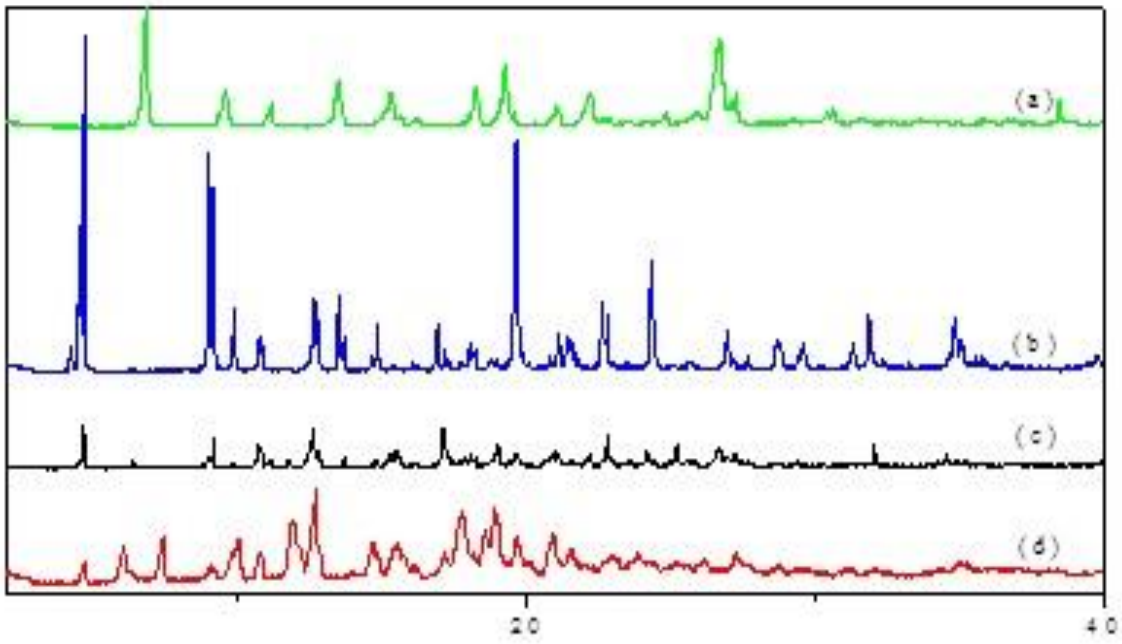


Fig. 3: Powder X-ray diffractometry of (a) ATB; (b) cyclodextrin; (c) physical mixture with molar ratio 1:1(guest:host)and (d) inclusion compound.

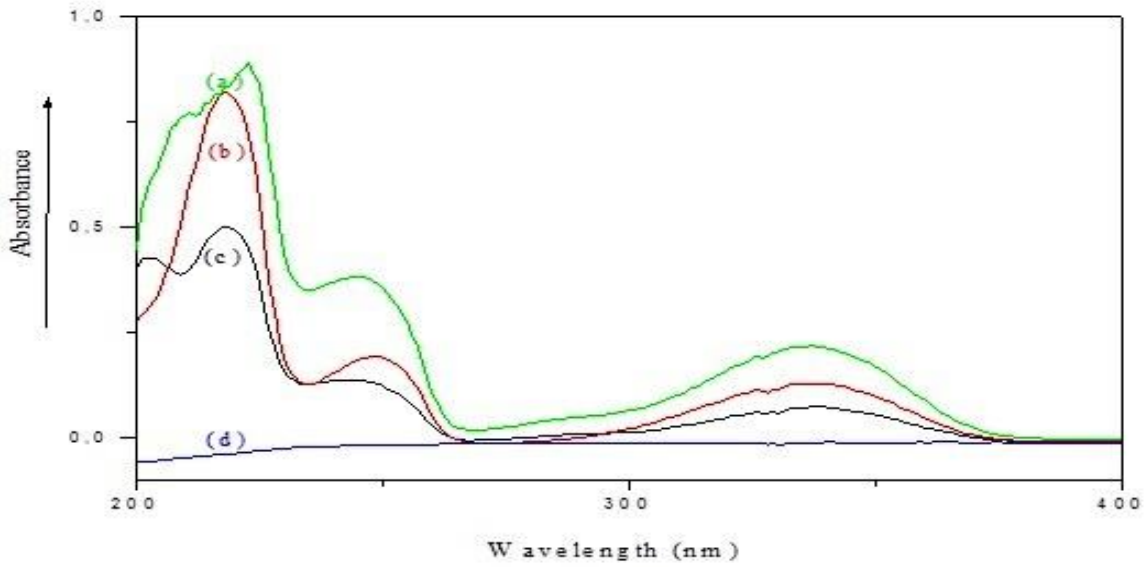


Fig. 4: UV spectra of (a) ATB; (b): physical mixture in molar ratio 1:1(guest:host); (c): inclusion compound and (d): cyclodextrin.

Table-1: Absorption spectral data of ATB and inclusion compound.

λ_{max}(nm)	A	ε (L. mol. cm^{-1}) 10^3
ATB	**245**	**1.182.6**
Physical mixture	**248**	**0.183.6**

FT-IR

The FT-IR spectrum of the complex is compared to that physical mixture; ATB and β-cyclodextrin. The differences between the spectra of ATB; beta-CD; physical mixture and complex confirm the formation of a new compound with different spectroscopic bands. The FTIR of the complex showed peaks at 3370-3500 cm^{-1} (hydroxyl and amine groups); 2937 cm^{-1} (Alkyl group); 1655 cm^{-1} (C=C group); 1279 cm^{-1} and 702.15 cm^{-1} (aromatic rings). Retention of most of the characteristic peaks of ATB was observed during the comparative analysis of the IR spectrum of ATB with the inclusion compound. The peak at 3500 cm^{-1} attributed to amine groups is visible in ATB; but it is modified greatly in inclusion compound (Table 2 summarizes the comparison of host; guest and complex wavenumber)).

We notice also on the FTIR spectrum a broad hydroxyl band of of in B-CD free at 3370 which has narrowed in the FTIR spectrum of inclusion compound which is a good indication of formation of complex. In addition, the FTIR spectrum of physical mixtures imitated the characteristic peaks of β-CD and ATB; which can be regarded as a simple superposition of those host and guest molecules.

Table-2: Comparison between wavenumber of beta-CD; ATB and inclusion compound of β -CD/ ATB.

Functional group	**νBeta CD**	**ν Inclusion complex β CD/ ATB**
ν_{OH}	**3370**	**3380**
ν_{CH2}	**2941**	**2935**
ν_{C-C}	**1157**	**1079**
Functional group	**νATB**	**ν Inclusion complex β CD/ ATB**
ν_{NH}	**3500**	**3410**
νC=O	**1668**	**1650**
νC=CHarom	**693**	**702**

NMR Spectroscopy

^{1}H NMR and ^{13}C NMR

^{1}H NMR

Nuclear magnetic resonance spectroscopy has been extensively employed in chemistry and can be considered as one of the most complete spectroscopic techniques due to its wide field of applications from structural elucidation to investigations on intra/inter-molecular. Applications of NMR to β-CD chemistry is so important that no other spectroscopic technique can provide the same wealth of chemical information on the supramolecular systems.

The simplest experiment NMR as an indicative of complexation is the observation of the difference in the proton chemical shifts between the free guest, the host species and the suggested complex. There has been a long time since the studies

on β-CD complexes were started by observing the chemical shifts changes of the protons H3 and H5 inside the cavity of β-CD [29].

Here, the ^{1}H-NMR spectra of the free ligands; ATB and the complex were studied in a mixture of $CDCl_3$+ DMSO-d_6; over the scan range 0 to 13 δ ppm for ^{1}H NMR. The chemical shifts (δ; in ppm) in both free and complexed states are reported in Table-3.

The complexation caused a variation in the chemical shifts of the β-CD and ATB protons and carbons; a large downfield shifts was recorded for H5 and H3 (Table-3). The NH shifted from 5.1 ppm to 5.53 ppm.

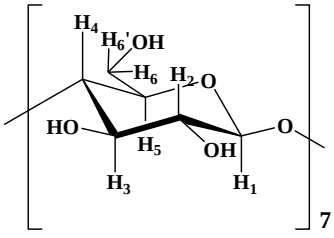
The variation of the shift was not the same for all protons. The minor modification observed for those at the exterior of the beta CD; depicted the existence of an interaction between the guest molecule and the interior of the host cavity; with a partial or complete inclusion on the torus; hence complexation. The H3 and H5 signal show, respectively, a downfield shift of about 0.032 ppm and 0.075 ppm. The downfiled shift observed for H5 and H3 confirms the inclusion inside the cavity. In addition Δδ H3 > Δδ H5; so a partial inclusion takes place [30].

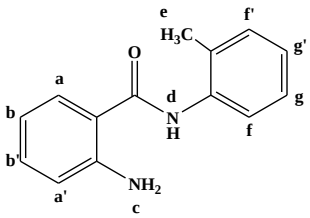
^{13}C NMR

The ^{13}C NMR spectra of ATB and ATB/ β -CD inclusion compound spectra confirm the formation of the inclusion compound. The experimental ^{13}C NMR chemical shifts are summarized in Table 4. Aromatic carbons give signals in overlapped areas of the spectrum with chemical shift values from 100 to 150 ppm. In our present investigation; the experimental chemical shift values of aromatic carbons were in the range 109.67-151.42 ppm. The experimental chemical shift values of β-CD carbons are in the range 59.89-101.90 ppm. As can be seen from Table 4.

Table-3: Chemical shifts, δ, and Δδ of protons of β-cyclodextrin in the free host; 2-Amino-N-o-tolyl-benzamide in free guest and inclusion compound

$\Delta\delta = \delta_2$ complex $-\delta_1$free guest.

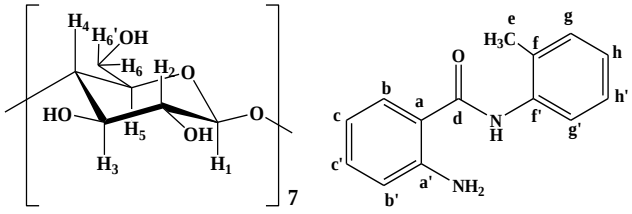




Protons	δ_1(ppm) of free compound	δ_2(ppm) upon complexation	Δδ en ppm
H_1	4.811	4.827	0.016
H_3	3.608	3.683	0.075
H_5	3.590	3.622	0.032
H6a;b	3.640	3.660	0.020
H_4	3.340	3.294	0.013
H_2	3.299	3.320	0.021
H_a(ATB)	7.800	7.650	-0.150
$H_{b'}$(ATB)	7.200	7.210	0.010
Hb(ATB)	6.800	6.500	-0.300
$H_{f;f'}$(ATB)	7.240	7.250	0.010
$H_{g;g'}$(ATB)	6.90	6.700	0.055
Ha'(ATB)	6.500	6.510	0.010
$H_{c;d}$(ATB) (mobile)	5.100	5.532	0.432
H_e(ATB)	2.40	2.48	0.080

Table-4: Chemical shifts δ and Δδ of protons of β-cyclodextrin in free host; 2-Amino-N-o-tolyl-benzamide in free guest and inclusion compound

$$\Delta\delta = \delta_2 \text{ complex} - \delta_1 \text{free guest}$$



Carbons	δ_1(ppm) of free compound	δ_2(ppm) upon complexation	Δδ en ppm
C_1	101.96	101.90	-0.06
C_3	72.14	72.00	-0.14
$C_{6:6'}$	59.87	59.89	0.02
C_5	73.40	73.01	-0.39
C_4	81.51	81.51	0.00
C_2	72.40	72.38	-0.02
$C_{a'}$(ATB)	150.80	151.42	0.62
$C_{f'}$ (ATB)	133.44	133.65	0.21
$C_{b'}$(ATB)	114.94	113.90	-1.04
C_g (ATB)	131.15	131.11	-0.04
C_f (ATB)	126.29	126.35	0.06
$C_{c'}$ (ATB)	129.76	129.80	0.04
C_c(ATB)	114.38	114.54	0.16
C_h (ATB)	116.03.	116.02	-0.01
$C_{g'}$(ATB)	110.08	109.67	-0.41
$C_{h'}$; (ATB)	123.12	123.11-0.01-	-0.01
C_d (ATB)	169.14	169.55	-0.400
C_e (ATB)	16.83	17.33	0.05
C_a(ATB)	114.9	114.54	0.400
C_b(ATB)	117.32	116.28	-1.040

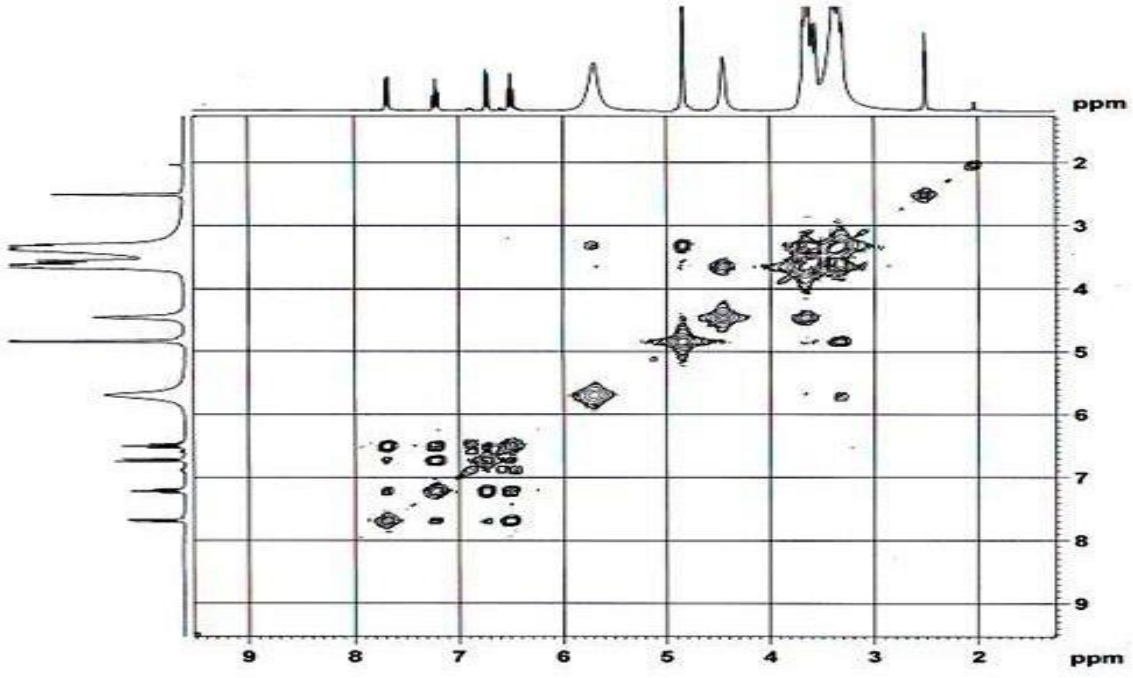


Fig. 5: NMR Noesy of inclusion compound β CD/ ATB.

The chemical shift of C=O shifted from 169.14 to 169.55.

The DEPT spectrum of ATB/β-CD inclusion compound gives the CH_2 peaks ($C_{6;6'}$) at 59.89 ppm. It can be seen that there is one CH_3 (C_e (ATB)) and at 17.34 ppm in the DEPT spectrum. The quaternary carbons, which disappeared in the DEPT spectrum, are not correlated to any protons.The other peaks of ^{13}C NMR, except for the peaks in the DEPT spectrum, are quaternary carbons;

2D NMR

Assignments of the protons and carbons were also made by two-dimensional homonuclear and heteronuclear correlated experiments. ^1H–^1H NMR; COSY; NOESY and HMBC spectra were studied in a mixture of $CDCl_3$+DMSO-d_6 respectively. The NOESY(Fig 5); HMBC(Fig 6) and COSY experiments, which are particularly useful for the determination of structural arrangements of molecular complexes, show the presence of the correlation between the protons (Hc; Hd); of NH and NH_2 with the two type of OH (4.80 and 4.23 ppm) of β-CD moiety. Hc and Hd overlap with H2; H3; H4; H5 and H6. The chemical shift of (Hc; Hd) located at 5.53 ppm; in addition there is no possible interaction between OH1;3;6 and H2; H3; H4; H5 and H6 of β-CD.

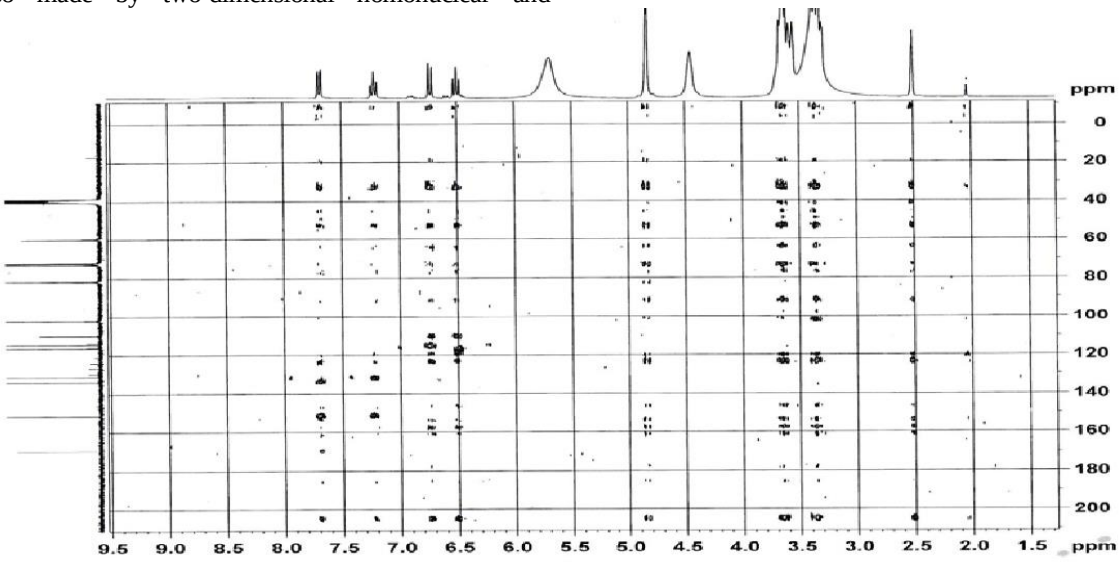


Fig. 6: HMBC spectra of inclusion compound β–CD/ ATB.

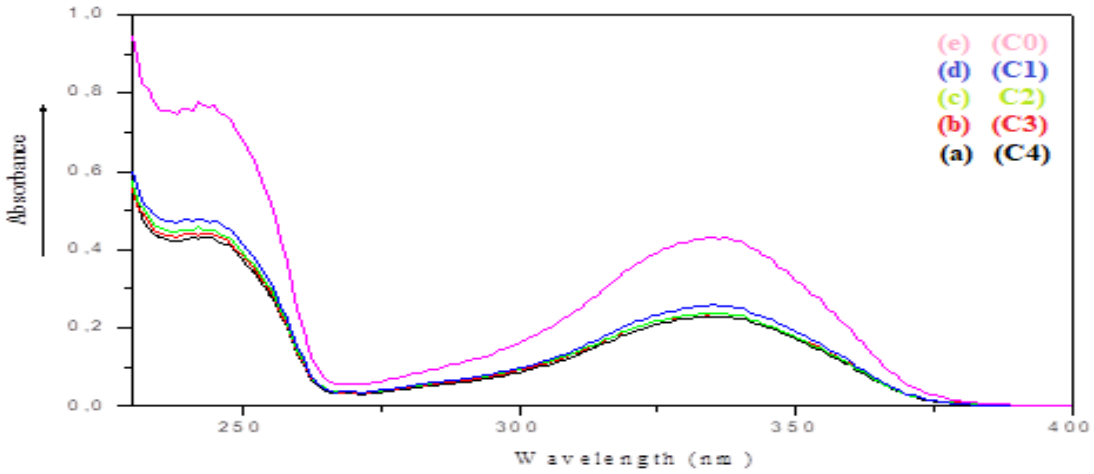


Fig. 7: Absorption spectra of ATB with various concentration of ß-CD.

Stoichiometry of the inclusion compound

Apparent formation constant and stoichiometry of the inclusion compound for ATB-β-CD inclusion compound.

The formation of complex between ATB and ß-cyclodextrin has been confirmed using UV-visible method. The stoichiometric ratio and binding constant of the ATB-ß-CD complex were obtained by measuring the changes in UV-visible of the absorbance of the ATB in the presence of increasing concentrations of the ß-CD. The concentration of ATB was maintained constant at 5 $x10^{-3}$ and the concentration of ß-CD was varied from 2.5 x 10^{-5} to 5 x 10^{-5}M.(Fig.12).The absorbance of solutions were measured at 243 nm. The absorption spectra of inclusion compound at different concentrations range of ß-CD is shown in Fig 8. It can be seen that solubility of ATB increased proportionally with an increase of concentration of ß -CD.

The stoichiometric ratio of the inclusion compound should be 1:1; if a linear relationship is obtained between 1/A vs. 1/[ß-CD] according the Hildebrand-Benesi Equation 1 [31])..

The Hildebrand-Benesi equation 1

$$1/A = 1/ \varepsilon[G]_0K[CD] + 1/ \varepsilon[G]_0$$

The good linear relationship obtained proved that stoichiometric ratio of the inclusion compound is 1:1. We estimated also the apparent formation constant from reciprocal plot for 1/*A vs.* 1/[ß-CD] with correlation coefficient *R*2 = 0.9922 The value of apparent formation constant was **2 x 10^2** mol.L^{-1}.

Solubility study

According to reported methods [32-33]; we added an excess (1 g) of ATB to 25 mL water containing various concentration of beta CD. The flasks have been stirred for two days. Then all suspensions were filtered through a 0.45 µm and assayed by UV spectrophotometer at 243 nm.The solvent evaporation allowed us to determine the ATB solubility at similar ratio for beta CD. ATB solubility was increased from 0.15 mg/ml to 3 mg /mL.

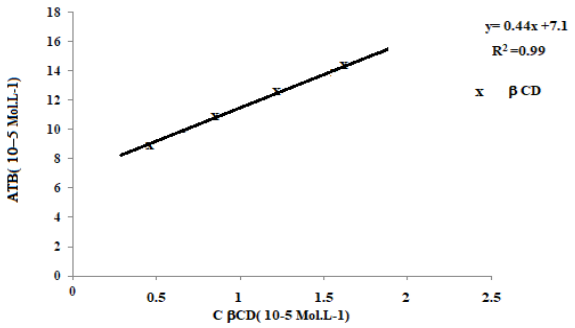


Fig. 8: Phase solubility diagram of ATB in aqueous solution of Beta CD.

Study of optical activity of inclusion compound

Optical activity measured in this work was realized by using a polarimeter which work with sodium lamp; Weight of every sample is m=9 x 10^{-3}g dissolved in 8 mL of Ethar ıl; temperature T= 20°C; length of the sample tube is 2ϭcm. The specific rotatory powers were $[\alpha]_D^{20°}$=162° for β-CD; $[\alpha]_D^{20°}$=93.3° for ATB and $[\alpha]_L^{20°}$=90.2° for inclusion compound. The inclusion of the achiral molecule of ATB in the β-cyclodextrin cavity generate a radical modification of the specific rotatory powers of the β-cyclodextrin. It is interesting to note that the spatial relationship between the center of symmetry; as well as the rigidity of the inclusion compounds; are of great importance for the character of the induced optical activity [34].

Study of Biological activity of inclusion compound

β-Cyclodextrin is the most used in pharmaceutical formulations due to its non-toxicity; biodegradability and reasonable cost. However; the application of unmodified β-CD is limited owing to its poor water solubility. The CDs and their inclusion compounds are always used as delivery systems in the drug; food [35-36].

Since then antioxidant research has received considerable attention and over a hundred thousand papers have been published on the subject. This has led to a rampant use of antioxidants in order to try to obtain and preserve optimal health. A number of food supplements are frequently fortified with synthetic or natural antioxidants.

However; the antioxidant activity does not automatically to a biological effect in vivo; due to the low solubility of ATB in water. This problem was minimized by using □-CD; which helps to increase solubility and protection against light induced decomposition and achieve controlled release of some compounds.

In vitro antioxidant activity

The objective of this study was to evaluate the antioxidant activity of new compounds a of the inclusion compound. The antioxidant properties were determined via the DPPH radical scavenging; the ABTS radical scavenging; ferric reducing power (FRP); hydroxyl radical scavenging and ferrous ion chelating activity (FIC).

The most abundant free radicals in biological systems are the oxygen-centered free radicals and their metabolites; usually referred to as ROS [37]. ROS are formed continuously as normal by-products of cellular metabolism; and; in low concentrations; they are essential for several physiological processes. However; when produced in excess; ROS can damage cell functionality as they can harm cellular lipids; proteins; and DNA [38]. Nowadays; interest is focused on the synthesis of new compounds with potential applications; such as cancer diagnosis and treatment of tumor [39].

DPPH radical scavenging activity

The free-radical scavenging activity of the various concentrations of new compounds (B-CD; ATB and Complex); and ascorbic acid was measured with the stable radical diphenylpicrylhydrazyl (DPPH) in terms of radical-scavenging activity.

The antioxidant activities of ATB; □-CD; ATB/ β-CD complex samples) were measured in terms of their radical scavenging ability using the DPPH method. 3 ml of DPPH (in methanol) was added to 100 µl of different compounds (dissolved in methanol); at different concentrations (1-0;5 mg/ml). After incubation 30 min; the absorbance measured at 517 nm according to a described procedure [40].

The result showed remarkable scavenging activity presented in Fig 9. The derive Complex had the highest capacity of scavenging DPPH radicals with percentage of inhibition 76.44 ±0.534% at the concentration (1mg/ml) and compared with ascorbic acid at the same concentration 91.26 ± 0.332%.

ABTS radical scavenging activity

ABTS assay was performed according to the protocol [41]. Differences for the ABTS•+ (2; 2-azobis-(3-ethylbenzothiozoline-6-sulphonic acid) radical scavenging capacities of each sample was recorded in Fig 10. The compound (Complex) was presented the highest capacity of scavenging at the high concentration (1mg/ml) with value 64.44± 0.74% and ascorbic acid at the same concentration 94.074±0.74%.

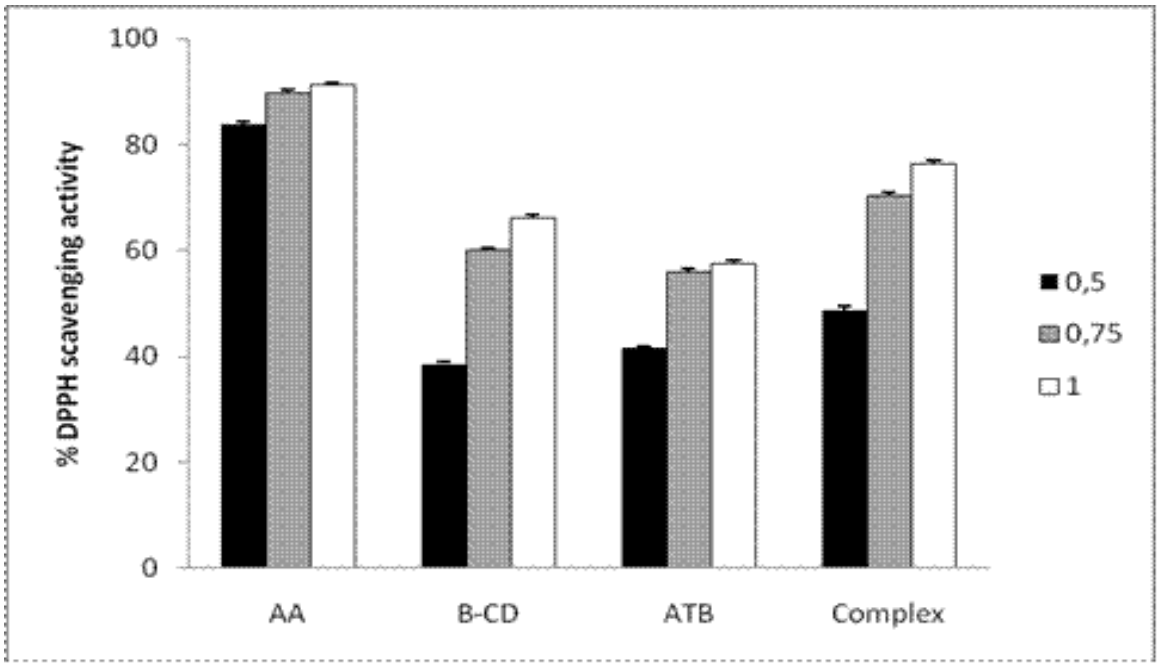


Fig. 9 DPPH Radical Scavenging Activity: tested compounds (B-CD; ATB and Complex); AA: ascorbic acid.

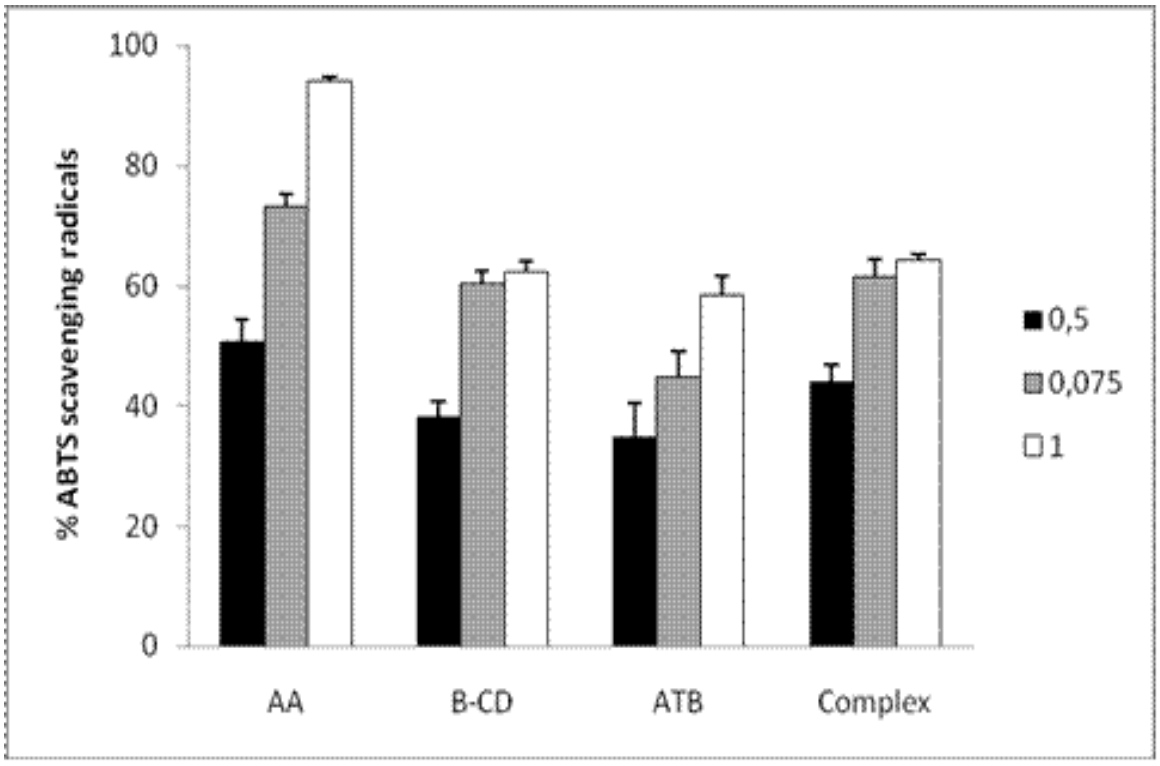


Fig. 10 ABTS radical scavenging ability: tested compounds (B-CD; ATB and Complex); AA: ascorbic acid).

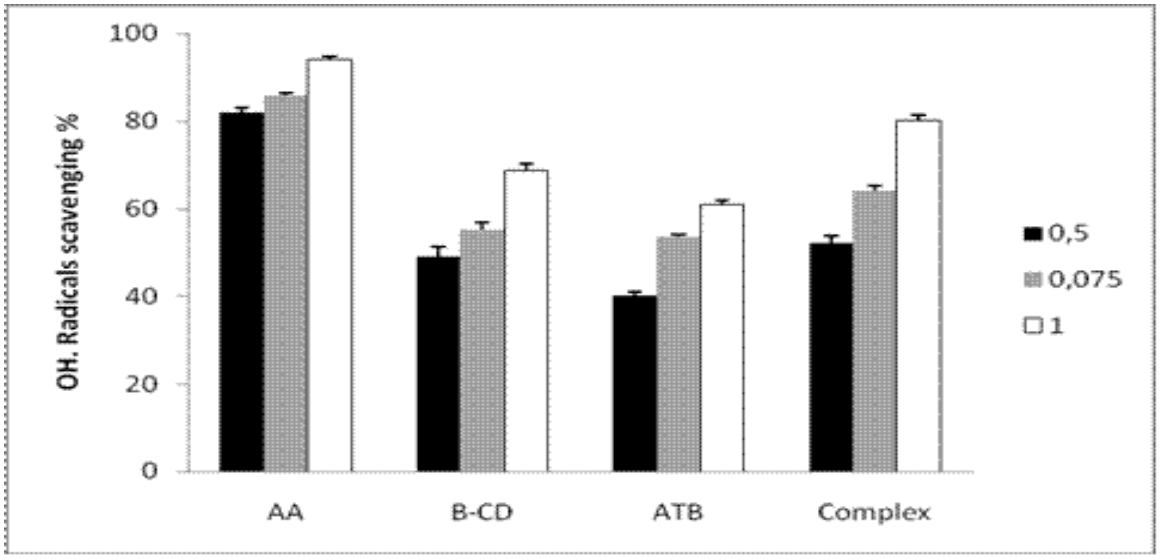


Fig. 11: OH radical scavenging ability: tested compounds (B-CD; ATB and Complex); AA: ascorbic acid).

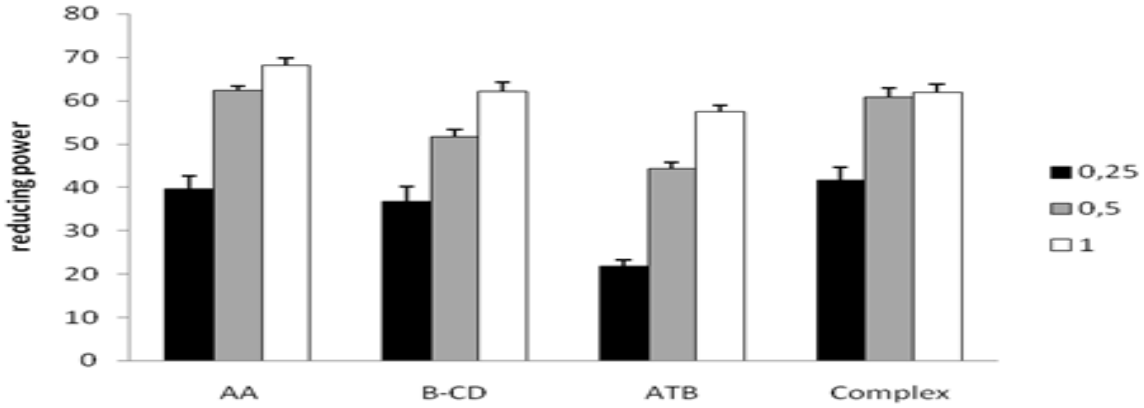


Fig. 12: Reducing power assay: tested compounds (B-CD; ATB and Complex); AA: ascorbic acid

Hydroxyl radical scavenging ability

The effect of compounds on hydroxyl radicals was assayed by using the deoxyribose method described by Halliwell and Gutteridge (1981) [42].

The results were summarized in the Fig 11 demonstrated that the most effective for hydroxyl radical scavenging activity followed by compounds complex 80.13 ± 1.318% and compared with ascorbic acid at the same concentration (1mg/ml) the percentage of inhibition was 94.01 ± 0.72 %.

Reducing propriety

The reducing power of new compounds (β-CD; ATB and complex) was assayed according to the method of Pulido et al (2000) [43]. The results was presented in Fig 12 reported that Complex has a high reducing property with percentage comparable to that of ascorbic acid and the percentage in the highest concentration (1mg/ml) is AA 68.22±1.55%; β-CD 62.21±1.92%; complex 61.97 ± 1.72%; and ATB 57.55 ± 1.26%.

Ferrous Ion Chelating (FIC) Ability

The extracts assessed for their ability to compete with ferrozine for iron (II) ions in free solution. The chelating ability of ferrous ions by various fractions was estimated by the method of Singh and Rajini [44].

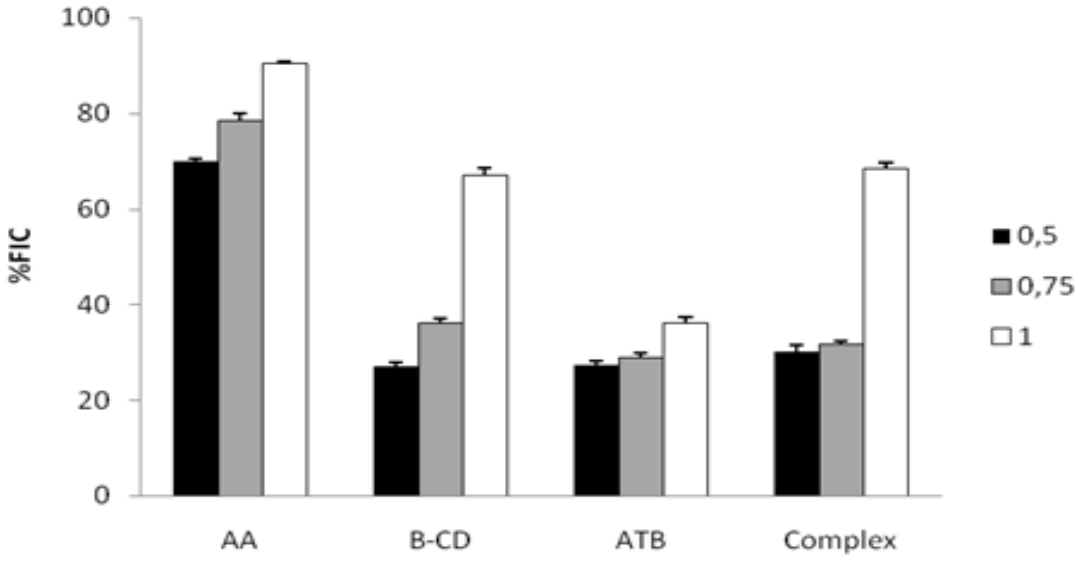


Fig. 13 Ferrous ion chelating (FIC) ability: tested compounds (B-CD; ATB and Complex); AA: ascorbic acid).

We reported in Fig 13; the ability of each compound in the different concentration to chelate ferrous ions. Our results showed that complex and β-CD at high concentration (1mg/ml) are the most compound have ability to chelating ferrous ions with percentage of chelating respectively 68.47±1.33% and 66.99±1.54% comparable to that of standard (ascorbic acid) at concentration (1mg/ml) FIC% = 90.4 ±0.36%.

Our results aimed to show the antioxidant activity in vitro of new compounds (β-CD; ATB and Complex). Our data showed that ATB and the complex have an antioxidant character; this is demonstrated by scavenging DPPH radicals; scavenging ABTS radicals; hydroxyl radical scavenging; reducing power and ferrous ion chelating ability significantly to that of ascorbic acid.

Conclusion

Formation of inclusion compound of ATB with β-CD by co-precipitation method enhanced considerably the solubility. FT-IR; ^{1}H NMR; 1H-1H NOESY; ^{13}C NMR; DEPT; HMBC and COSY spectra study suggested the presence of interaction between ATB and β-CD in inclusion compound. FTIR and XRD have different characteristics when compared with free ATB; thus ATB was efficiently complexed in the beta CD. In addition a specific rotatory powers data results suggest the formation of a stable complex of ATB and β-CD. UV-visible method indicates the formation of 1:1 stoichiometric inclusion compound. The apparent complexation constant ($K_{1:1}$) calculated is 2 x 10^2 L /mol at room temperature. The solubility study; confirm that solubility is increased by complexation. The antioxidant activities of ATB and ATB/-CD complex were measured using the stable free radical and the results are shown in Fig 14. As shown in Fig 14; when the concentration of the ATB and inclusion compound was 0;25-1 mg/mL; the DPPH scavenging activity; OH radical scavenging ability; Reducing power and the Ferrous ion chelating (FIC) ability of the ATB/ □-CD complex was higher than those of the ATB. These results confirm that the antioxidant activity of ATB was significantly increased by the formation of the inclusion compound with Beta CD.β

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References

1. Y. N. Mabkhot, A. M. Al-Majid, A. Barakat, S. S. Al-Showiman, S. A. Munirah, R. Smaail, N. Muhammad Moazzam, and B. H. Taibi, Synthesis and Biological Evaluation of 2-Aminobenzamide Derivatives as Antimicrobial Agents: Opening/Closing Pharmacophore Site, *Int. J. Mol. Sci*., **15**, 5115 (2014).
2. A. Kumar Singha, M. Lohanib and R. Parthsarthya, Synthesis, Characterization and Anti-Inflammatory activity of Some 1, 3,4 - Oxadiazole Derivatives, *Iran. J. Pharm. Res. Spring***., 12**, 319 (2013).
3. O. Moradei, S. Leit, N. Zhou, S. Fréchette, I. Paquin, S. Raeppel, F. Gaudette, G. Bouchain, S. H. Woo, A. Vaisburg, M. Fournel, A. Kalita, A. Lu, M. C. Trachy-Bourget, P. T. Yan, J. Liu, Z. Li , J. Rahil, A. R. MacLeod, J. M. Besterman and D. Delorme, Substituted N-(2-aminophenyl)-benzamides, (E)-N-(2-aminophenyl)-acrylamides and their analogues: novel classes of histone deacetylase inhibitors, *Bioorg. Med. Chem. Lett*., **16**, 4048 (2006).
4. A. Pîrnău, M. Bogdan, and G. F. Călin, NMR spectroscopic characterization of β-cyclodextrin inclusion complex with vanillin, *J. Physics*: Conference Series., **182**, 012 (2009).
5. Z. Yanmei, Z. Haifeng, L. Sensheng, P. Danwei and S. Guanshuo, Water as a hydrogen source in palladium-catalyzed reduction and reductive amination of nitroarenes mediated by diboronic acid, *Tetrahedron.*, **73**, 3898 (2017).
6. C. Danyi, Z. Yanmei, Z. Haifeng, L. Sensheng, L. Qixing, Z. Kaili and U. Yasuhiro, Metal-free Reduction of Nitro Aromatics to Amines with $B_2(OH)_4/H_2O$, *synlett*., **29**, 1765 (2018).
7. C. Randall Clark, L. Ching-Ming, and R. T. Sansom, Synthesis and biological activity of benzothiazolo[3,2-a] quinolone antibac, *J. Med. Chem*., **29,** 1534 (1986).
8. I Javed, SZ Hussain, I Ullah, I Khan, M Ateeq, G Shahnaz, H ur Rehman, Synthesis, Characterization and Evaluation of Lecithin Based Nanocarriers for Enhanced Pharmacological and Oral Pharmacokinetic Profile of Amphotericin B, *J.Mater.Chem. B.*, **3** (42), 8359 (2015).
9. R Nazir, M. Mazhar, M.J. Akhtar, M.R. Shah, N.A Khan, M. Nadeem, N. Siddique, M. Mahmoud, N.M.Butt, Superparamagnetic bimetallic iron–palladium nanoalloy: synthesis and characterization, *Nanotechnology*., **19**, 185608 (2008).
10. A. Lateef, R. Nazir, N. Jamil, S. Alam, R. Shah, M.N. Khan, M. Saleem, S-ur. Rehman, Synthesis and characterization of environmental friendly corncob biochar based nano-composite – A potential slow release nano-fertilizer for

sustainable agriculture, *Environ. Nanotechnol. Monit. Manag*., **11**, 100212 (2019).

11. R. Iacovino, F. Rapuano, J. Valentina Caso, A. Russo, M. Lavorgna, L. Russo, G. Malgieri and C. Isernia, β-Cyclodextrin Inclusion complex to Improve Physicochemical Properties of Pipemidic Acid: Characterization and Bioactivity Evaluation, *Int. J. Mol. Sci.*, **14**, 13022 (2013).
12. S. Milin, S. Vatsal, G. Anasuya, Z. Zheng and M. Tamara, Molecular Inclusion complexes of β-Cyclodextrin Derivatives Enhance Aqueous Solubility and Cellular Internalization of Paclitaxel: Preformulation and In vitro Assessments , *J. Pharm. Pharmacol.*, **10**, 2, 8 (2015).
13. N. Rajendiran, R.K. Sankaranarayanan and J. Saravanan, Nanostructures formed by cyclodextrin covered aminobenzophenones through supramolecular self assembly, *Spectrochim. Acta*., **127,** 52 (2014).
14. H. Hamdi, R. Abderrahim, and F. Meganem, Spectroscopic studies of inclusion complex of β-cyclodextrin and benzidine diammonium dipicrate, *Spectrochim. Acta. A*., **75**, 32 (2010).
15. N. Rajendiran, T. Mohandoss and G. Venkatesh, Investigation of inclusion complexes of sulfamerazine with α- and β-cyclodextrins: An experimental and theoretical study, *Spectrochim. Acta. A.*, **124**, 441 (2014).
16. S. Siva, J. Thulasidhasan and N. Rajendiran, Host–guest inclusion complex of propafenone hydrochloride with α- and β-cyclodextrins: Spectral and molecular modeling studies, *Spectrochim. Acta., A.*, **115**, 559 (2013).
17. F. Jara, C. Mascayano, M. Caroli Rezende, C. Tirapegui, A. Urzua, A spectral and molecular dynamics simulation study of β-cyclodextrin inclusion complexes with solvatochromic dyes derived from barbituric acid, *J. Incl. Phenom. Macrocycl Chem.*, **54**, 95 (2006).
18. N. Rajendiran and S. Siva. Inclusion compound of sulfadimethoxine with cyclodextrins: Preparation and characterization, *Carbohyd Polym*., **101**, 828 (2014).
19. N. Rajendiran and R.K. Sankaranarayanan, Azo dye/cyclodextrin: New findings of identical nanorods through 2: 2 inclusion complexes, *Carbohyd Polym*., **106**, 422 (2014).
20. J. Wang, Z. Qiu, Y. Wang, Li. Li, X. Guo, D.T. Pham, S. F. Lincoln and R. K. Prud'homme, Supramolecular polymer assembly in aqueous solution arising from cyclodextrin host–guest complexation, *Beilstein*., **12,** 50 (2016).
21. B. Medronho, A. J. M. Valente P, Costa and A. Romano, Inclusion complexes of rosmarinic acid and cyclodextrins: stoichiometry, association constants, and antioxidant potential, *Colloid. Polym. Sci*., **292**, 885 (2014).
22. T. Loftsson and D. Duchêne, Cyclodextrins and their applications, *Inter. J. Pharm*., **329**, 1 (2007).
23. C. Machut, J. Patrigeon, S. Tilloy, H. Bricout and F. Hapiot, Monflier, E. *Self-Assembled Supramolecular Bidentate Ligands for Aqueous Organometallic Catalysis,* Angew. *Chem. Int. Ed.*, **46**, 3040 (2007).
24. J. Szejtli, Introduction and General Overview of Cyclodextrin Chemistry, *Chem. Rev*., **98**, 1743 (1998).
25. O. Aleem, B. Kuchekar, Y. Pore and S. Late, Effect of β-cyclodextrin and hydroxypropyl β-cyclodextrin complexation on physicochemical properties and antimicrobial activity of cefdinir, *J. Pharm. Biomed. Anal*., **47**, 535 (2008).
26. X. Wen, F. Tan, Z. Jing and Z. Liu, Preparation and study the 1:2 inclusion complex of carvedilol with beta-cyclodextrin, *J. Pharm. Biomed. Anal*., **34**, 517 (2004).
27. M. Zhao, H. Wang, B. Yang and H. Tao, Identification of cyclodextrin inclusion complex of chlorogenic acid and its antimicrobial activity, Food Chem., **120**, 1138 (2010).
28. M. Banchero, S. Ronchetti, and L. Manna, Characterization of Ketoprofen/Methyl-*β*-Cyclodextrin Complexes Prepared Using Supercritical Carbon Dioxide, *J. Chem.*, **2013**, 8 (2013).
29. M. Poorghorban, A. H Karoyo, P. Grochulski, R. E. Verrall, L.D. Wilson and I. Badea, A 1H NMR Study of Host/Guest Supramolecular Complexes of a Curcumin Analogue with β-Cyclodextrin and a β-Cyclodextrin-Conjugated Gemini Surfactant, *Mol. Pharm*., **12**, 2993 (2015).
30. P. Farras, H. Waller and A. C. Benniston, Enhanced Photostability of a Ruthenium(II) Polypyridyl Complex under Highly Oxidizing Aqueous Conditions by Its Partial Inclusion into a Cyclodextrin, *Chem. Eur. J*., **22**, 1133 (2016).
31. H. A. Benesi, and J. H. A. Hildebrand, Spectrophotometric Investigation of the Interaction of Iodine with Aromatic Hydrocarbons, *J. Am. Chem. Soc.*, **71**, 2703 (1949).

32. J. Xu, Y. Zhang, X. Li and Y. Zheng, Inclusion complex of nateglinide with sulfobutyl ether β-cyclodextrin: Preparation, characterization and water solubility, *J. Mol. Struct*., **1141**, 328 (2017).
33. J. Szejtli, Highly Soluble β-Cyclodextrin Derivatives, Starch. *Stärke*., **36**, 429 (1984).
34. F. Hirayama, and K. Uekama, *Methods of investigating and preparing inclusion complexes,in: D. Duchene (ed.), Cyclodextrins and Their Industrial Use*s, Editions de Santé, Paris., 133 (1987).
35. Li. Shujing, Li. Yuan, Bing. Zhang, Wei. Zhou, Xinrui. Wanga and Dongsheng. Bai., Photostability and antioxidant activity studies on the inclusion complexes of *trans*-polydatin with β-cyclodextrin and derivatives, *RSC. Adv*.**, 8**, 25941 (2018).
36. M. Del Valle, Cyclodextrins and their uses, Proc. *Biochem.*, **39**, 1033 (2004).
37. R. Peramaiyan, N. Natarajan, R. Thamaraiselvan, P. Rajendran, N. G. Edwinoliver, L. Uppalapati, G. Jacob and N. Ikuo, Antioxidants and human diseases, Clinica. *Chimica. Acta*., **436**, 332 (2014).
38. N. Sugino, Roles of reactive oxygen species in the corpus luteum, *Anim. Sci J*., **77**, 556 (2006).
39. B. Vivekanand, K. Mahendra Raj and B. H. M. Mruthyunjayaswamy, Synthesis, characterization, antimicrobial, DNA-cleavage and antioxidant activities of 3-((5-chloro-2-phenyl-1*H*-indol-3-ylimino)methyl)quinoline-2(1*H*)-thione and its metal complexes, *J. Mol Struc*.,**1079**, 214 (2015).
40. A. Braca, N.D. Tommasi, L.D. Bari, C. Pizza, M. Politi and I. Morelli, Antioxidant Principles from Bauhinia tarapotensis, *J. Nat. Prod*., **64**, 892 (2001).
41. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yong and C. Rice-Evas, Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free. *Rad. Biol Med***., 26**, 1231 (1999).
42. B. Halliwell, J. M. C. Gutteridge and O. I. Aruoma, The deoxyribose method: a simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals, *Anal. Biochem.,* **165,** 215 (1987).
43. R. Pulido, L. Bravo and F. Saura-Calixto, Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay, *J. Agric. Food Chem.*, **48**, 396 (2000).
44. N. Singh and P.S. Rajini, Free radical scavenging activity of an aqueous extract of potato peel, *Food Chem*., **85**, 611 (2004).