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Spectrophotometric studies of a novel Gedunin-2-Hydroxypropyl-β-cyclodextrin binary system

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Abstract

Background: Gedunin, a limonoid, is linked with antimalarial, anticancer and anti-allergic activities. This study was aimed at preparing an inclusion complex of gedunin and 2-hydroxypropyl-β-cyclodextrin (HBD) to increase solubility of gedunin in polar solvents which will increase absorption and bioavailability *in vivo* and thus enhance pharmacological effects.

Materials and methods: Gedunin was obtained from the hexane extract of *Entandrophragma angolense* heartwood by column and preparative thin layer chromatography. The structure was previously confirmed by spectroscopic means (NMR). The electronic absorption spectra data of the complexes formed between gedunin and HBD in various solvents was determined using the UV-VIS spectrophotometer. The stoichiometry of inclusion was determined by Job's method of continuous variation.

Results: Evidence of interaction was observed between gedunin and HBD in the various solvents but gedunin and its complex with HBD exhibited sharp absorption bands in acetate buffer (pH 3.5). The spectrophotometric titrations showed curves with a single point of inflexion when the experiment was carried out at 25^0C (298 K) and 37^0C (310 K). A stoichiometric ratio of 1:1 for complex formation was obtained. The formation constants (K_f) obtained at 25^0C and 37 ^{0}C were 9.539 x 10^3 M^{-1} and 1.853 x 10^4 M^{-1} respectively. Thermodynamic considerations revealed hydrophobic interaction between gedunin and HBD.

Conclusion: A stable inclusion complex of gedunin and HBD was formed at room and body temperature. This complex formation involved trapping of poorly soluble gedunin into the hydrophobic core of the cyclodextrin and may enhance the pharmacological activity of gedunin *in vivo*.

Keywords: *Gedunin, 2-hydroxypropyl-β-cyclodextrin, Inclusion complexes, Spectrophotometry, Thermodynamic considerations*

Résumé

Contexte: Le gédunin, un limonoïde, est liée aux activités antipaludiques, anticancéreuses et antiallergiques. Cette étude avait pour but de préparer un complexe d'inclusion de gédunin et de 2-hydroxypropyl-β-cyclo-dextrine (HBD) pour augmenter la solubilité dugédunin dans des solvants polaires qui augmentent l'absorption et la biodisponibilité in vivo et par conséquence d'améliorer les effets pharmacologiques.

Matériels et méthodes: Le gédunin a été obtenu à partir de l'extrait d'hexane d'Entandrophragmaangolense duramen par colonne etpréparation en couche mince de la Chromatographie. La structure était confirmée préalablement par des moyens spectroscopiques (RMN). Les données des spectres d'absorption électroniques des complexes formés entre le gédunin et HBD dans différents solvants ont été déterminées en utilisant le spectrophotomètre UV-VIS. La stœchiométrie d'inclusion a été déterminée par la méthode de Job de variation continuée.

Résultats: Preuve d'interaction a été observée entre le gédunin et HBD dans les différents solvants, mais le gédunin et son complexe avec HBD présentait des bandes d'absorption nettes dans un tampon d'acétate (pH 3,5). Les titrages spectrophotométries ont montré des courbes avec un point d'inflexion unique lorsque l'expérience a été effectuée à 25^0C (298 K) et 37^0C (310 K). Un rapport stœchiométrique de1: 1 pour la formation du complexe a été obtenu. Les constantes de formation (K_f) obtenus à 25^0Cet 37 ^{0}C étaient 9,539 x10^3 M^{-1}et 1,853 x 10^4 M^{-1}respectivement. Les considérations thermodynamiques ont révélé une interaction hydrophobique entre le gédunin et HBD.

Conclusion: Un complexe d'inclusion stable de gédunin et HBD a été formé à la températuredе salle et du corps. Cette formation de complexe a impliqué le piégeage des gédunins peu soluble dans le noyau hydrophobiqu e de la cyclo-dextrine et peut augmenter l'activité pharmacologique de gédunin in vivo.

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Mots-clés: *Gédunin, 2-hydroxypropyl-β-cyclodextrine, Complexes d'inclusion, Spectrophotométrie, Considérations thermodynamiques*

Introduction

Plants from the Meliaceae family are widely used for the treatment of fever in traditional medicine in tropical countries [1-2]. A study carried out by McKinnon *et al* [3] showed that the most common denominator in the Meliaceae was the presence of limonoids, in particular, gedunin. Gedunin and some of its derivatives have been reported to possess a wide range of biological activities. These are antimalarial [4], anti-feedant, insecticidal [5-8], antifungal [9], anti-prostate cancer [10-11], antileishmanial [12], anti-HIV and as inhibitor of colon cancer cells [11,13]. Others include antiproliferative [14], anti-secretory [15], antifilarial [16] and anti-allergy [17]. Mahmoodin, a derivative of gedunin, has been reported to possess antibacterial activity [18]. The neurotrophic effects of deoxygedunin in mice [19] and the anti-inflammatory activity of 7-deacetyl gedunin have also been reported [20].

Gedunin has been reported to be potent *in vitro* against *Plasmodium falciparum.* It had an IC_{50} of 0.8 µg/mL against the chloroquine-resistant K1 strain of *P. falciparum*, this was roughly equivalent to that of quinine [21]. In another study, it had an IC_{50} of 0.70 µg/mL and was the most potent among twenty seven limonoids tested against the same strain of *P. falciparum*. This activity was about three times higher than chloroquine but twenty times lower than quinine [22]. McKinnon *et al*. [3] reported that gedunin had better activity than chloroquine against *P. falciparum* chloroquine resistant (W2/Indochina) clone. Gedunin was the most active of five limonoids isolated from *K. grandifoliola* (IC_{50} of 1.25 µg/mL) against the chloroquine resistant W2 clone. It was equally active against chloroquine-sensitive and chloroquine-resistant *P. falciparum* clinical isolates used in the study [23]. Despite its *in vitro* antiplasmodial activity, an *in vivo* study of gedunin administered orally or subcutaneously to mice infected with *Plasmodium berghei*, a murine malaria parasite, in a 4-day test resulted in no inhibition of parasitaemia [22]. This has been partly attributed to poor solubility and low uptake due to its lipophilicity, first pass metabolism by intestinal cytochrome P-450 enzymes of the small intestines, which reduces its plasma levels and hydrolysis to its inactive and unstable metabolite, 7-deacetylgedunin [24].

Bioavailability of poor water soluble drugs has been improved by several techniques which are designed to increase the aqueous solubility of the drugs. Solid dispersion, solvent deposition, micronization, addition of surfactants, modification of drug crystal forms, co-precipitation with polymers and addition of cyclodextrins are some of such techniques. Micronization in water was employed in solubilizing gedunin by Bray *et al*. [22] but this resulted in a lack of *in vivo* antimalarial activity. The co-precipitate of gedunin with polyvinyl pyrollidone can only be dispersed, but not dissolved, in water. When orally administered at 50 mg kg^{-1} day^{-1} for four days, this formulation was able to suppress the parasitaemia level by 44 % when compared to no suppression of parasitaemia observed with the water-based preparation of gedunin used in the earlier study. However, no clear dose-response effects were observed in the 0-100 mg kg^{-1} day^{-1} dose range [2].

One other method of solubilization is by complexation with polymers such as cyclodextrins which are non-toxic macrocyclic biodegradable oligosaccharides that contain at least 6 D-(+) glucopyranose units attached by α-(1, 4) glucosidic bonds. They have a relatively hydrophobic central cavity and a hydrophilic outer surface. Cyclodextrins and their derivatives have the capability to form non-covalent inclusion complexes both in solution and in solid state with a wide variety of guest molecules [25,26]. The formation of inclusion complexes favorably alter physical, chemical and biological properties of guest molecules which are surrounded by the hydrophobic environment of the cyclodextrin cavity [26-28]. Therefore, a reduction in variability in oral drug absorption can be achieved through enhancement of drug dissolution rate as a result of improved aqueous drug solubility by cyclodextrin complexation [26, 29]. Furthermore, complexation can increase the stability of the therapeutic substance preventing degradation or bioconversion at the absorption site [26, 30]. Other advantages of cyclodextrin complexation include masking of bad taste or colour, reduced side effects and the possibility of an improved drug release system for better bioavailability.

Gedunin derivatives and their structure activity relationship have been studied. The preparation of these semi-synthetic derivatives usually involves lots of steps and complicated chromatographic separations. However, it has been reported that none of these derivatives is more active as antimalarial and antiproliferative agent in *in vitro* studies [3, 14]. 7-methoxygedunin which has 50 % of its *in vitro* antiplasmodial activity is about twice more active than gedunin *in vivo* because it is more stable to degradation

thus a promising two-in-one approach which might improve the solubility and stability of gedunin *in vivo* is the preparation of gedunin-cyclodextrin binary systems. No attempt made at enhancing the aqueous solubility of gedunin by formulation of binary systems with cyclodextrins has been reported. It was thought worthwhile to investigate this possibility by forming an inclusion complex of gedunin and 2-hydroxypropyl-β-cyclodextrin. The chemical structures of both gedunin (GED) and 2-hydroxypropyl-β-cyclodextrin (HBD) are presented in Fig. 1. This cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (HBD), a hydroxyalkylated β-cyclodextrin, has been widely used in pharmaceutical applications because of its high water solubility and solubilizing power, and low toxicity compared to the parent cyclodextrin, β-cyclodextrin [26].

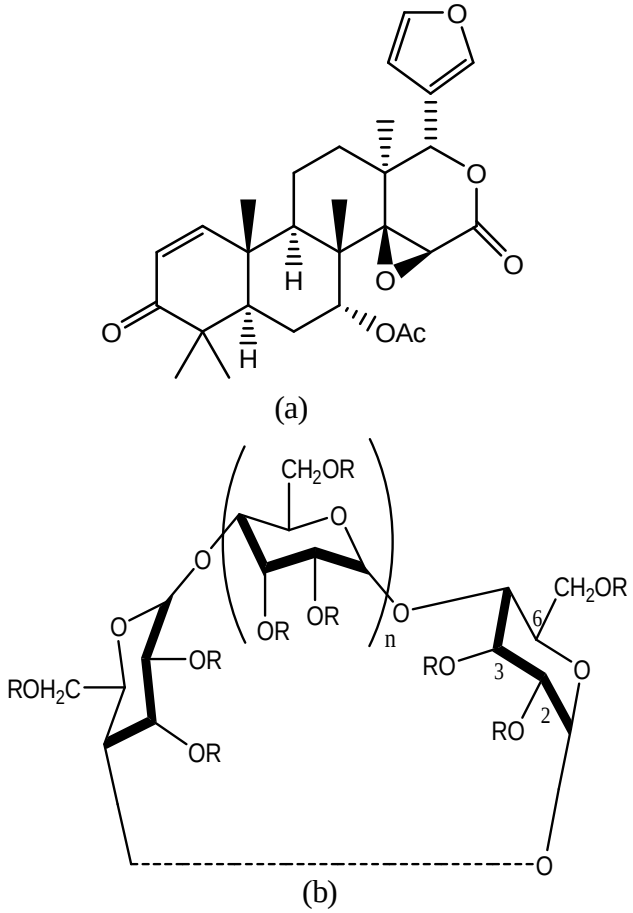

Fig. 1: Structures of Gedumin (a) and hydroxylpropyl-β-cyclodextrin [R=$CH_2CHOHCH_3$ or H and n=5] (b)

In this study, an attempt was made at preparing an inclusion complex of gedunin and HBD with the aim of improving the solubility of GED in polar solvents. It is expected that increased solubility will enhance its bioavailability and pharmacological actions.

Materials and methods

Apparatus

Spectrophotometric measurements were carried out with a UV-Visible spectrophotometer (Perkin Elmer, USA).

Materials

Gedunin was isolated from *Entandrophragma angolense* Welwitsch C.D.C (Meliaceae) heartwood as described by Okhale *et al*. [31]. 2-hydroxypropyl-β-cyclodextrin was purchased from Sigma-Aldrich Chemical Company (Japan). Ethanol, ammonium acetate, boric acid, potassium hydroxide and sodium hydroxide pellets, were of analytical grade obtained from BDH, Poole, England and distilled water was used throughout the experiments.

Determination of electronic absorption spectra data of the complexes formed between Gedunin and 2-hydroxypropyl-β-cyclodextrin in various solvents.

Equimolar solutions (0.5 mM) of GED in 95 % ethanol and HBD in distilled water were prepared. 4 mL of each of the following solvents: 95 % ethanol, ethanol: water (1:5), acetate buffer pH 3.5, phosphate buffer pH 6.8, phosphate buffer pH 7.5 and borate buffer pH 9.6 were added to 1 mL of the GED solution (0.5 mM) in separate test-tubes. The solutions were left at room temperature for 20 minutes. The final solutions were then scanned to obtain their absorption spectra using the UV-VIS spectrophotometer in the wavelength range of 190 – 700 nm. The spectral data for the HBD was acquired by adding 4 mL of each of the solvents listed above to 1 mL of the HBD solution in separate test-tubes and the electronic absorption spectra was obtained within the same wavelength range.

Determination of stoichiometric ratio for the complex formation

The stoichiometry of inclusion was determined by the method of continuous variation developed by Job [32]. Equimolar solutions of GED and HBD were mixed in separate test tubes and making the final volume up to 1 mL thereby varying the molar concentration to obtain the exact combination ratio. The molar ratios adopted for the HBD were 0, 0.1, 0.2, 0.25, 0.33, 0.5, 0.67, 0.75, 0.8, 0.9 and 1.0. The solutions were allowed to stand for 30 minutes before 4 mL of acetate buffer pH 3.5 was added to each of them. The absorbance readings for each sample solution were recorded at 245 nm. The difference in absorbance in the presence

and in the absence of HBD was plotted against the molar ratio of the host species (HBD). The stoichiometric ratio experiment was carried out at two different temperature levels, 25 ^{0}C (298 K) and 37 ^{0}C (310 K).

Results

Electronic absorption spectra in various solvents

The electronic absorption spectral data (wavelengths of maximum absorbance, λ_{max} and the corresponding molar absorptivity values, log ε_{max}) of gedunin and the complexes formed by gedunin with 2-hydroxylpropyl-β-cyclodextrin in various solvents are presented in Table 1 and Figure 2. Figure 2 shows lack of significant light absorption by 2-hydroxypropyl-β-cyclodextrin in all the solvents adopted. Overlaid absorption spectra of GED and the complex formed between gedunin and cyclodextrin (GCD) in ethanol: water (1:5) and absolute ethanol are as shown in Fig. 3. Gedunin in ethanol: water exhibited three absorption bands in the wavelength range adopted. These bands consist of strongly absorbing peaks at 195 and 230 and a weak band at 315 nm. On binding to HBD for the complex formation, all the three bands experienced slight hypochromic shifts without any change in the wavelength of absorption. For gedunin in ethanol, two bands were observed at 220 and 320 nm which depicts significant bathochromic shifts relative to hydro-alcoholic mixture. On interaction

Table 1: Electronic absorption spectral data of the complexes formed by Gedunin (GED) with hydroxylpropyl-β-cyclodextrin (HBD) in various solvents

	λ_{max}, nm (log ε_{max})					
	Solvents					
Sample	ETW	ET	P1	P2	B	Ac
GED	195 (4.142)	220(4.111)	195 (3.909)	200 (3.939)	195(3.933)	245(3.823)
	230 (4.110)	320 (1.958)	230 (4.118)	230 (4.111)	225(4.103)	325(2.432)
	315 (2.626)		320(3.251)*	315 (3.314)	315(2.578)	
GECD	195 (4.112)	225 (4.092)	195 (3.829)	195 (3.828)	195(3.864)	245(3.700)
	230 (4.103)	310(2.370)	230 (4.061)	230 (4.071)	225(4.079)	325(1.990)
	315 (2.308)		315(2.848)*	325 (2.568)	320(2.012)	

**shoulder at this wavelength*

Abbreviations: ETW (Ethanol: water; 1:5), ET (Absolute ethanol), P1 (Phosphate buffer pH 6.80), P2 (Phosphate buffer pH 7.50), B (Borate buffer pH 9.60), Ac (Acetate buffer pH 3.50), GED (Gedunin), GECD (Gedunin-HBD complex)

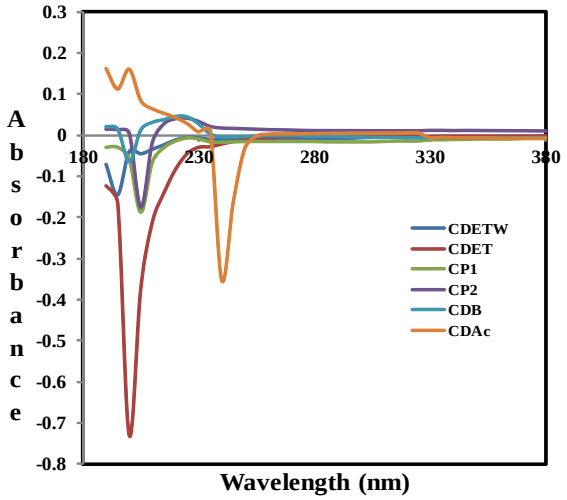

Fig. 2: Absorption Spectrum (UV region) of cyclodextrin in ethanol: water (1:5), absolute ethanol, phosphate buffer (pH 6.80), phosphate buffer (pH 7.50), borate buffer (9.60) and acetate buffer (pH 3.50)

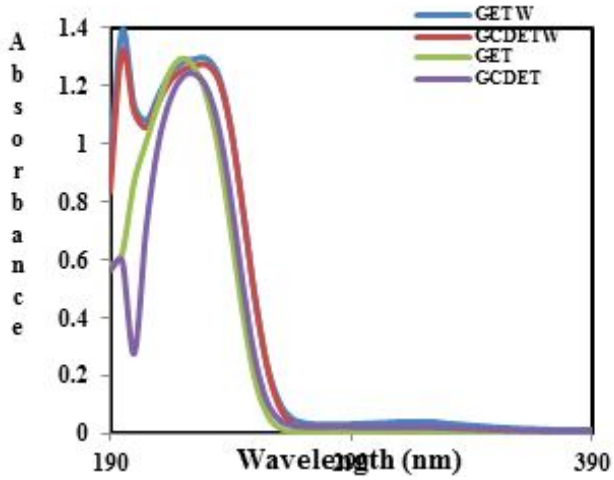

Fig. 3: Overlaid absorption spectra of Gedunin (GED) and the complex formed between Gedunin and Cyclodextrin (GCD) in ethanol: water (1:5) and absolute ethanol.

with HBD, the two bands experienced slight bathochromic shifts accompanied by hypochromic shifts. However, in spite of the bathochromic shifts, the absorptivities of the two bands in absolute ethanol were lower relative to that recorded in hydro-alcoholic mixture.

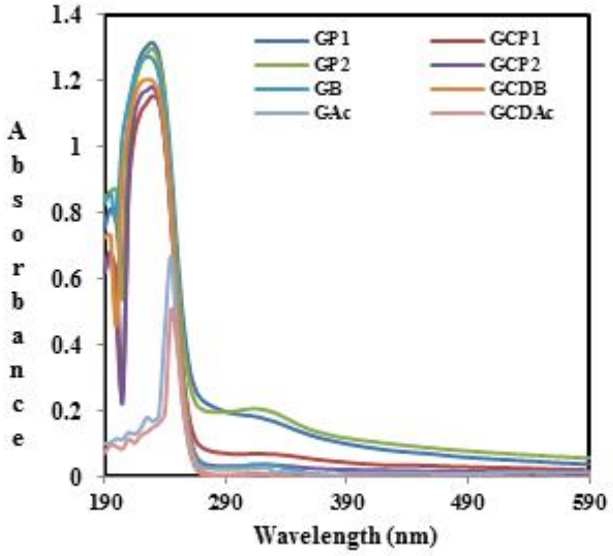

Fig. 4: Overlaid absorption spectra of Gedunin and the complex formed between gedunin and cyclodextrin in buffers (P1 =Phosphate 6.80, P2 = Phosphate 7.50, B = Borate 9.60 and Ac = Acetate 3.50)

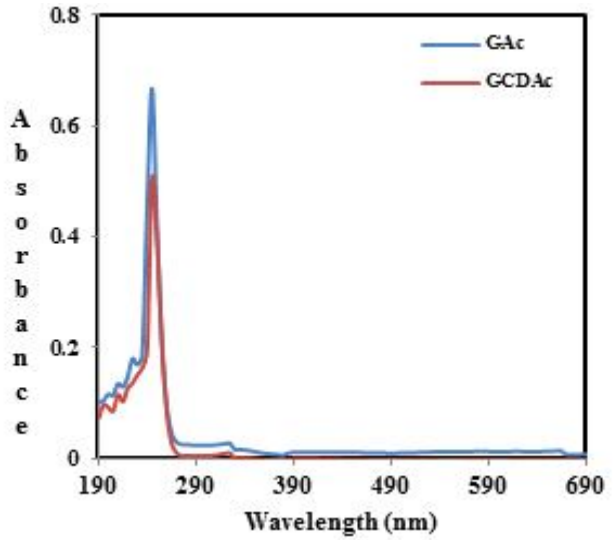

Fig. 5: Overlaid absorption spectra of Gedunin and the complex formed between Gedunin and cyclodextrin in acetate buffer pH 3.50

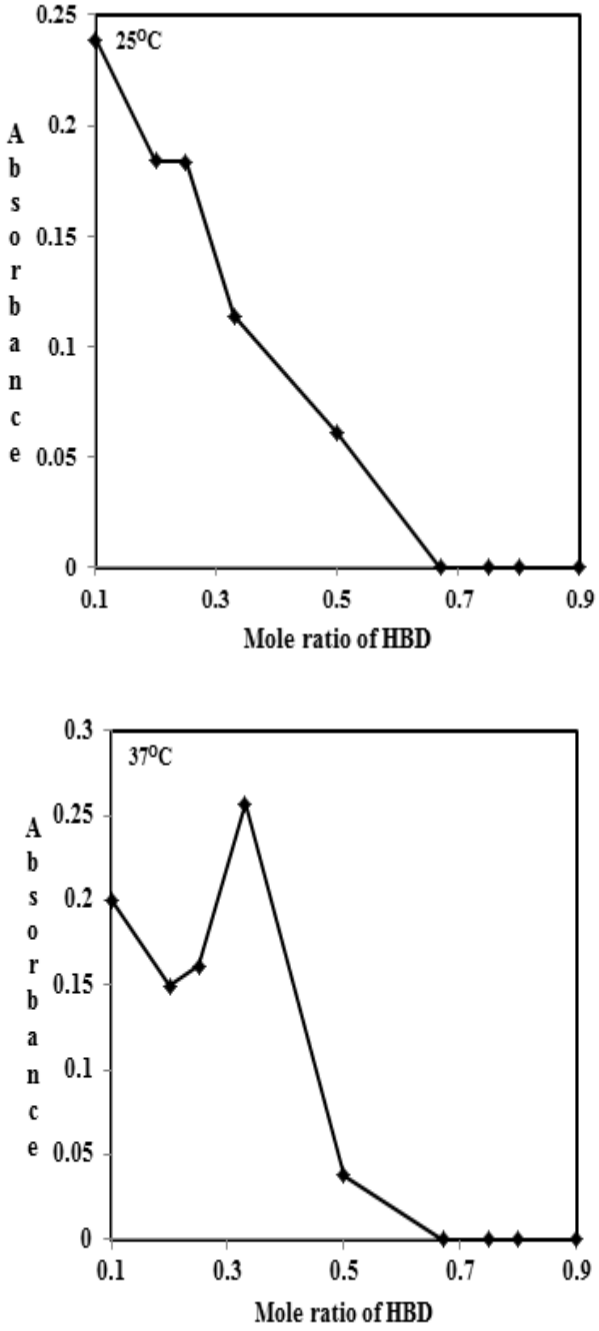

Fig. 6: Determination of stoichiometric ratio for complex formation between GED and HBD at 25 and 37 ^{O}C

The observed patterns of gedunin and gedunin complex with HBD in different buffers are shown in Figure 4 while the electronic absorption data are shown in table 1. Two clearly defined absorption peaks are observed for both gedunin and the complex in phosphate buffer pH 6.80, while the third peak occurred as a shoulder for the two species. The bands occurring at 195 and 230 nm in gedunin were unchanged on forming

the complex. These bands were however associated with pronounced hypochromic shifts in both cases. The shoulder presented with a slight hypsochromic shift ($\lambda = \Delta\lambda$ nm) though also with pronounced hypochromic shift. The shoulder observed for gedunin in phosphate buffer 6.80 at 315 nm disappeared to an identifiable peak at 320 nm on increasing the buffer pH to 7.5. In all three absorption bands observed at this pH (200, 230 and 315 nm) for gedunin, significant hypsochromic shifts were again observed on forming the complex with HBD. A further increase in pH to 9.60 resulted in a not too drastic wavelength change with significant hypochromic shifts on gedunin complex formation with HBD. In acetate buffer (Figure 5), the only significant peak for gedunin occurred at 245 nm with a minor peak at 325 nm. On forming a complex with HBD the main peak experienced a hypochromic shift though the absorption peak is maintained. The minor peak at 325 nm was also associated with a pronounced hypochromic shift.

Determination of stoichiometric ratio

The assessment of stoichiometric ratio was done using the Job's method of continuous variation [32]. The assessments were conducted both at 25 ^{O}C and 37 ^{O}C. The results are presented in Figure 6. In both instances, a single point of inflection was observed showing a mole of GED combines with 1 mole of HBD.

a UV-VIS spectroscopic investigation was carried out. The extent of interaction was studied by recording the absorption spectra of the intact drug and after its complex formation with HBD in various solvents. Other solvents, apart from water, were selected based on the initial study that revealed lack of significant aqueous solubility of GED in water, thus preventing complex formation with HBD. The solvents adopted were ethanol: water mixture (1:5) and absolute ethanol. The influence of pH changes was studied by the application of buffers of varying pH values as media for the investigation of complex formation. The buffers utilized in the study are phosphate buffer (pH 6.80), phosphate buffer (pH 7.50), borate buffer (pH 9.60) and acetate buffer (pH 3.50). Since the native GED is practically insoluble in aqueous media for the probability of forming a complex with HBD, these various solvents were adopted in order to promote ionization and exposure of any likely latent functional groups that can aid in the interaction of the two pairs. From the GED structure (Fig. 1), functional groups that can be exposed and aid the formation of ion-ion induced interactions are present in rings B and D as well as the furan moiety.

The absorption spectra of HBD, GED and the complexes formed in the various solvents are presented in Figures 2-5. Figure 2 reveals lack of significant light absorption by the cyclodextrin in all the solvents adopted.

Table 2: Formation constants and thermodynamic parameters for the GED-HBD complex formation

	Formation constant, K (M^{-1})		Free energy change, $-\Delta G$ (KJ mol^{-1})		ΔH (KJ mol^{-1})	ΔS (KJ mol^{-1})
	298 K	310 K	298 K	310 K		
GED-HBD complex	9.539×10^{3}	1.853×10^{4}	22.702	25.327	+ 42.453	+ 0.066

Table 3: Variation of Massieu-Plank function with formation constant

Complex	298 K		310 K	
	Massieu-Plank function	Log K_f	Massieu-Plank function	Log K_f
GED-HBD	0.0762	3.9795	0.0817	4.2679

Discussion

Electronic absorption spectra in various solvents

In order to gain insight into the binding or otherwise of the drug candidate, GED with the host species, HBD, This is not unexpected as there are no extensive chromophores within the HBD skeleton (Fig. 1).

The slight absorption observed in the buffers at wavelength ranges 200 - 225 nm must be due to the interaction of the hydroxyl groups with the buffers. The

exposure of these latent hydroxyl groups in HBD by the buffers is attested to by the lack of absorption peaks in the other solvents especially absolute ethanol and the hydro-alcoholic mixture. HBD exhibited negative peaks in all the other solvents except some minor absorption peaks in acetate (220 nm), borate (220 nm) and phosphate pH 7.5 (225 nm). This implies that adequate interactions with GED will be made possible for complex formation. Majority of the cyclodextrins are basic in nature (Tang and Tang, 2013) with *pKa* values of about 12.33, 12.2 and 12.08 respectively for α-, β- and γ-cyclodextrins. The values confer on the cyclodextrins the ability to interact with buffers in the acidic range as well as those in weakly basic ranges. These explain why there was absorption spectral changes in the presence of acetate buffer, borate and phosphate buffers.

The behaviours of GED and its complexes in the solvents utilized in this study were completely different from that observed for HBD. The overlaid absorption spectra of GED and the complex formed with HBD in hydro-alcoholic mixture (ethanol: water; 1:5) and absolute ethanol are presented in Fig.3. The presence of water in the hydro-alcoholic mixture presented the opportunity for a preferential solvation of the complex by the mixture which is not possible in absolute ethanol. This might account for the hypsochromic shifts observed in ethanol- water mixture relative to ethanol alone. In fact, the high energy band at 195 nm completely disappeared in absolute ethanol. For the hydro-alcoholic mixture, ionization and preferential distribution of the GED in the two miscible phases may provide an opportunity for enhanced interaction with the HBD. This in turn has completely eliminated the problems associated with extreme hydrophobicity of GED and allowing for its solubilisation by HBD.

The buffers adopted in the study of the light absorption patterns of GED and the inclusion complex were purposively selected with a wide-ranging pH values. The phosphate buffers were selected in order to mimic the biological system where eventual absorption and distribution will take place while the borate and acetate buffers were selected to observe the mode of interactions at two extremes of basic and acidic regions of the pH scale.

The observed interaction of GED with HBD in phosphate buffer 6.80 (Figure 4) appears to be related to some ion-ion interactions since either molecule can ionize at the pH of the buffer. Since the interaction did not produce any bathochromic shift or significant hypsochromic shift for the first two absorption bands, a stacking of the molecules for interaction might be occurring. This is opposed to ionic bond interaction or charge transfer complexation that would have resulted in some significant bathochromic shift due to formation of a new chemical entity with extended chromophoric elongation. Thus, other binding interactions such as hydrophobic bond may be prominent.

The influence of increasing the pH of the medium of interaction to 7.50 led to slight changes in the observed spectral patterns. The slight bathochromic shift of the shoulder at 315 nm to an identifiable peak at 325 nm and the significant hypochromic shifts of the absorption bands at 200, 230 and 315 nm, on forming the complex with HBD, further corroborates the earlier observation that a new molecule is not produced rather an association of some type occurs.

The absorption spectral pattern in borate buffer pH 9.60 however appears slightly different from that of pH 7.50 but significant hypochromic shifts were again recorded. This observation led to the investigation of the behaviours of GED and HBD in acetate buffer pH 3.50 (Fig. 5). Sharp bands were observed in the absorption spectra of both GED and the complex. The two peaks at 245 nm and 325 nm experienced a hypochromic shift though the absorption peak is maintained. This implies that the interaction with an acid allowed for the formation of discrete bonds not involved in extensive solute-solvent interactions that usually produce band broadening in UV spectrum. The presence of an acidic medium also aids ionization of cyclodextrins since they all possess basic pKa values. The formation of very sharp bands between GED and HBD in acetate buffer led to the adoption of acetate buffer as the medium for the further studies on the interaction between the two compounds.

Spectrophotometric Titrations

Determination of stoichiometric ratio

The quantitative investigation of the inclusion complexation behaviour between GED and HBD was carried out by studying the stoichiometric ratio at which the interaction took place using the Job's method of continuous variation. The spectrophotometric titrations were carried out by using equimolar concentrations of GED and HBD (0.5 mM) in acetate buffer pH 3.50. The observed stoichiometric ratio determination at 298 K is presented in Figure 6a. The influence of temperature on the interaction between GED and HBD

was studied using the body temperature at 310 K (Figure 6b). The temperatures were carefully selected in order to observe the interaction at both room temperature and normal body temperature to decipher possibility of altered binding following temperature change. In both instances, the curve generated a single point of inflection which points to the formation of a 1:1 mole ratio between GED and HBD. However, for all mole ratio combinations attempted, the absorptivities were higher at 310 K compared to 298 K. The implication is that the reaction must be some endothermic reaction which will require elevated temperatures to proceed fast to completion. Thus, it is anticipated that the interaction in a biological system will have a higher propensity of occurrence than at room temperature.

Previous studies with the cyclodextrins have shown that the size/shape-fit concept plays a crucial role in the formation of inclusion complexes of host cyclodextrins with guest molecules of various structures [33]. On the basis of this size/shape-fit concept, several intermolecular forces such as ion-dipole, dipole-dipole, van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions are known to cooperatively contribute to the inclusion complexation. Since the two compounds did not interact in their native state, the ionic interaction was induced by the adoption of various buffers as stated above. The stability of the host-guest complex will then be greatly influenced by the type of the intermolecular binding forces adopted by the molecules in their interaction with each other. In particular, insertion of GED within the hydrophobic cavity of HBD is expected to give rise to specific stability constants which can be used in justifying the formation or otherwise of a stable complex.

Estimation of formation constants

The binding of GED with the HBD macromolecule is hypothesized to proceed as an inclusion of the GED molecule within hydrophobic core of the HBD. The formation of a stable complex will be associated with the extrusion of water of hydration from the host surroundings. The ability of the extruded water molecules to remain without perturbing the inclusion complex is greatly influenced by the formation constant. The energy required to drive such bond formation will be provided by the magnitude of the formation constant. Equilibrium is envisaged to exist between the inclusion complex and free forms of GED and HBD. From the equilibrium expression, the formation constant can be estimated by the knowledge of molar absorptivities of the free and bound GED molecules. For small molecules binding to active sites on a macromolecule, the formation or binding constant can be estimated from the expression in Eq. (1) [34-37].

$$\frac{A_o}{A-A_o} = \frac{\varepsilon_a}{\varepsilon_{H-G}-\varepsilon_G} + \frac{\varepsilon_a}{\varepsilon_{H-G}-\varepsilon_G}\frac{1}{K_f}\frac{1}{[HBD]}$$

(1) Where A_o and A are the absorbances of free and bound GED respectively

ε_G is the molar absorptivity of free GED and $\varepsilon_{H\text{-}G}$ is that of the complex K_f is the formation constant.

A straight-line trend was obtained which is the typical Benesi–Hildebrand plot for the estimation of the formation constant of molecular complexes [34]. From the plot of *Ao*/(*A*-*Ao*) against 1/[HBD], the ratio of the intercept to the slope gives the formation constant, K_f. The formation constants obtained at the two different temperatures are presented for the complexes in Table (2). At room temperature, the formation constant was $9.539 \times 10^3\ M^{-1}$ while that of 310 K was $1.853 \times 10^4\ M^{-1}$. Thus, elevating the temperature of the medium at which the interaction took place led to an increase in the formation constant. This further corroborates the enhanced absorptivity observed in the determination of the stoichiometric ratio.

Thermodynamic considerations

The thermodynamic considerations of the interactions of GED with HBD is important as it will give an insight into the type and extent of forces that are operating to give the observed effects outlined above in the considerations of the spectral behaviors, binding constant and mole ratio properties of the inclusion complex formed between the pair. As observed earlier the formation of inclusion complexes by the cyclodextrins proceed with the utilization of such weak secondary binding forces as ion-dipole, dipole-dipole, van der Waals, electrostatic, hydrogen bonding and hydrophobic interactions individually or cooperatively to generate a stable complex.

The thermodynamic parameters, free energy change (G), enthalpy (ΔH) and entropy (ΔS) of interaction can be used to delineate the type of forces operational in the binding of biomoloecules to small molecule substrate. To gain an understanding of the type of forces in this study, the interaction of GED with HBD was carried out at two temperature levels and the thermodynamic parameters were estimated from the knowledge of the formation constant.

The standard free energy change for the formation of the intermolecular complex was estimated using Eq. (2), where R is the gas constant, T is the temperature in Kelvin and K_f is the formation constant (M^{-1}).

$$-\Delta G = RT\ln K_f \quad (2)$$

The free energy change as a function of temperature is presented in Table 2. The negative values of ΔG reveal that the interaction process is spontaneous. A higher value was obtained at 310 K showing that the spontaneity of the formation of the inclusion complex is aided by elevated temperature. The implication of this is that higher temperature might favor an increased solubility of the GED which in turn will enhance its aqueous solubility.

The enthalpy change (ΔH) and the entropy change (ΔS) were estimated from a modified Van Hoff's equation for two temperatures. The formation constants at the two temperature levels considered in this study were used to estimate the enthalpy change Eq. (3) while the entropy change was estimated from Eq. (4).

$$\ln\left\{\frac{K_2}{K_1}\right\} = \frac{-\Delta H^0}{R}\left\{\frac{1}{T_2} - \frac{1}{T_1}\right\} \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

The estimated thermodynamic parameters are presented in Table 2. The enthalpy change (ΔH) is positive giving a value of + 42.45 KJ mol^{-1} while the entropy change for the inclusion complex gave a small positive value of + 0.066 KJ mol^{-1}.

For transfer of small molecules from polar to non-polar environments, hydrophobic interactions usually give ΔH>0and ΔS>0 with negative ΔG [38]. In order to study temperature effects on thermodynamic stability, a modified consideration of ΔG^0 values with formation constant was attempted as earlier adopted by Schellman [39] to study the aqueous solubility of hydrocarbons. This consideration examines the effect of temperature on stability when related to hydrophobic interaction. Correlation between $\Delta G^0/T$ with log K_f provided a better estimate of how hydrophobic interactions can predict thermodynamic stability rather than the use of ΔG^0 alone. The variation of $\Delta G^0/T$ (Massieu– Planck function) with log K_f is presented in Table 3. The values obtained as presented in Table 3 shows that there is a positive correlation between the Massieu-Planck function and log K_f thereby confirming that the formation of inclusion complex between GED and HBD involved the trapping of the poorly soluble GED into the hydrophobic core of the cyclodextrin. This will lead to an enhancement of the apparent aqueous solubility of GED, i.e. enhanced solubilization.

Conclusion

Gedunin formed a stable non-inclusion complex with HBD in the presence of acetate ions. The stoichiometric ratio for inclusion complex formation between gedunin and 2-hydroxypropyl-â-cyclodextrin at 25 ^{0}C and 37 ^{0}C was found to be 1:1. The solubility of gedunin in polar solvents was improved and this should make it more physiologically available as well as improving its pharmacological activity. Knowledge of the interaction between the gedunin and HBD may provide useful information on the development of its pharmaceutical dosage forms for oral administration.

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