KINETICS, THERMODYNAMICS AND ANTIOXIDANT ACTIVITIES OF WATER AND ETHANOL EXTRACT OF STEM BARK *OF ANACARDIUM OCCIDENTALE.*

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(Received: 12th Feb, 2015; Accepted: 28th July, 2015)

ABSTRACT

Extraction is a required process for the utilization of bioactive compounds from their natural sources as ingredients and additives in food, pharmaceutical and cosmetic industries. Extracts from various parts of *A. occidentale* have received massive investigations especially in the management and treatment of various ailments which include diabetes, malaria, yellow fever as well as diarrhoea. In this study, the stem bark of *A. occidentale* was extracted with water and ethanol. Antioxidant activities of the extracts were determined using DPPH, hydrogen peroxide free radical scavenging and ferrous ion chelating activities. To explain the solid–liquid extraction processes of *A. occidentale* in water and ethanol, the kinetic of extraction in ethanol was best fitted with pseudo first order while that in water fitted best with the second order kinetic models. The isotherm fitted well with Power law models for ethanol and water extraction process. The transition rate theory was used to estimate the thermodynamic parameters, the $\Delta H^{\#}$ values of 49.67 and 47.37 kJ mol^{-1} obtained for the ethanol and water extraction processes respectively showed that water extraction process is endothermic while ethanol extraction is an exothermic process. The $\Delta S^{\#}$ values of -0.09 and -0.42 kJ $mol^{-1}K^{-1}$ obtained for the ethanol and water extraction processes respectively suggest a higher entropy in ethanol than in the water extraction processes.

Keywords: Extraction; Pseudo first order; second order; Isotherm; Thermodynamic;

INTRODUCTION

Extraction is an essential process in the transformation of active compounds from their natural sources into active ingredients and additives in food, pharmaceutical and cosmetic industries. Factors such as temperature and solvents nature are the extensively considered for process efficiency and product quality (Liu *et al.* 2000).

Evaluation of the extraction process involves kinetic analysis, incorporating mathematics and experimentation for the assessment of the parameters that affects the efficiency of the extraction. Kinetic models may be physical or empirical; while the physical models are based on the physical phenomena of mass transfer through plant particles and from external solid surfaces into bulk of the liquid phase (Kitanovic´ *et al.* 2008), the empirical models offer limited insight into the fundamental principles involved. However, it provided an excellent basis for curve fitting and allowing representation as a function of physical properties and process conditions of both the extraction medium and the extracting plant material. It is a useful engineering tool for an objective, fast and economic assessment of any process.

Various extraction models for the description of extraction process and the assessment of parameter effects on the extraction efficiency and product quality are available in literature. Differences in these models (even in the identical models) can be attributed to the variations in target compounds and structures of natural sources as well as type of processes (Karacabey *et al.*, 2013). Therefore, it is necessary to be established independently for any system and condition where its use is considered. The mass transfer mechanism of bioactive compounds from the interior of plant materials to the bulk of the liquid extract can be simply explained with two stages involving rapid washing of free target solute from the plant particles and slow diffusion of solute through plant material; the latter one is usually the rate-limiting step of the overall process (Gertenbach, 2002; Kitanovic´ *et al.* 2008).
Anacardium occidentale is a tree in the family Anacardiaceae with about 73 genera and 600 species (Correa, 1978). The fruit is used as food while extracts from roots, stems and leaves have

been used for medicinal purposes (Sokeng, *et al.* 2001). Recently, extracts from various parts of *A. occidentale* had been massively investigated in the management and treatment of various ailments which include but not limited to diabetes, malaria, yellow fever as well as diarrhea (Abdullahi and Olatunji, 2010). Also, with anti-microbial effects against micro-organisms like *Staphylococcus aureus, Salmonella typhi, Websiella pneumonia, Escherichia coli* (Vijayakumar and Kalaichelvan, 2011; Gonçalves and Gobbo, 2012). Free radical scavenging has harnessed the antioxidant ability of not only cashew leaves extract (Jaiswal *et al.,* 2010; Chermahini *et al.,* 2011; Fazali *et al.,* 2011) but also the seed coats of its nut (Vijayakumar and Kalaichelvan, 2011).

Basically, water and ethanol are two main solvents used in the extraction process of cashew plant, acetone has also been experimentally proven to yield better results. However, the usage of acetone-based extracts are limited due to its poisonous effect, hence they cannot be used as food additives or ingredient in pharmaceuticals (Chaves, *et al.* 2010; Vijayakumar and Kalaichelvan, 2011; Gonçalves and Gobbo, 2012).

The important aspects for performance evaluation of solid-liquid extraction process are mainly based on the experimental kinetics of the extraction process. Estimation of effective diffusivity of the extract from the plant matrix plays an important role in extraction kinetics. To explain the solid–liquid extraction processes of *A. occidentale* in water and ethanol, the experimental data were fitted with four empirical models including time as an independent model variable: first-order, second order kinetic models, Peleg's model, intra-particulate diffusion model and Power law equation in order to determine the best model. This will provide useful information for the initial sizing and the economic evaluation of the system in a commercial scale. Antioxidant activities of the extracts were also investigated by free radical scavenging activity of the extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH).

MATERIALS AND METHODS

Plant Material

The barks of the tree plant were collected from the cashew farm at the Federal University of Agriculture, Abeokuta. They were dried at room temperature and ground into powder with a milling machine. The ground bark was sieved into a particle size of 30 – 50 μm and stored as dried powder in an air-tight container until needed.

Proximate Composition

Proximate composition of the samples namely, moisture, ash, crude protein, carbohydrates and crude fibre were determined according to the Association of Official Analytical Chemist (AOAC; 1990) methods. The results were expressed in wet basis. All analyses were done in duplicate and averaged.

Chemical Reagents

The reagents used are all analytical grade from British Drug House, London and they include Ethanol, Methanol, H_2O_2, $FeSO_4$, NaOH and HCl 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Aldrich chemical. Water used for reagent preparations and extraction is doubly distilled against borax glass.

Extraction Procedure

Fifty grams (50 g) of powdered plant material was extracted in 500 ml of distilled water using soxhlet extraction apparatus. The extraction was carried out for 5 hours and the extract was thereafter concentrated by evaporation in a rotary evaporator.

Phytochemical Screening

The ethanol and water extracts of *Anacardium occidentale* tree bark were screened for phytochemical constituents which include: Alkaloids, Saponnins, Flavonoids and poly phenolics, Tannins, Terpenoids and Steroids; following standard methods (Sofowora, 1993; Harbone, 1998; Trease and Evans, 2002).

Determination of Antioxidant Activity

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging

The antioxidant activity of the extract was measured in terms of hydrogen-donating or radical scavenging ability, using the stable radical, DPPH according to the method of Nickavar *et al.* (2006). This was studied using different concentrations of the extract in methanol. A

methanolic stock solution (1 ml) of the extract (0.03 – 1.0 mg/ml) in a 5 ml flask, 0.2 ml of 0.6138 mM methanolic solution of DPPH was added. It was kept in the dark and absorbance measured after 30 min at wavelength of 517 nm on a UV/visible spectrophotometer (T60U, make). The absorbance of the DPPH radical without extract was measured as control. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula (Yen *et al.*1993):

$$\% \ inhibition = \frac{(A_{co} - A_{A(t)}) \times 100}{A_{c(o)}}$$

where A_{co} is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at any time t (t= 30 min free radical inhibition study).

Hydrogen Peroxide (H_2O_2) Radical Scavenging Assay

Hydrogen peroxide scavenging activities of the extracts were determined according to the method described by Ruch et *al.* (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Aqueous solutions of the extract (1-10 µg/ml) were added to the hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide and compared with ascorbic acid, the reference compound.

$$\% \ H_2O_2 scavenged = \frac{(A_{co} - A_{A(t)}) \times 100}{A_{co}}$$

Where, A_{co} is Absorbance of the control and $A_{(A)(t)}$: is Absorbance of the extracts/standard

Ferrous Ions Chelating Activity

Ferrous ion chelating activities of the extracts were measured as described by Yamaguchi *et al.* (1995). 1ml $FeSO_4$ solution was mixed with extract of different concentration. 1ml Tris HCl buffer (pH 7.4) and 2,2'-bipyridyl solution were added together with hydroxylamine – HCl and ethanol respectively. The reaction mixture was adjusted to a final volume of 5 ml with distilled water, shaken well and incubated for 10 minutes at room temperature. Absorbance was determined at 522 nm and percent chelation was calculated as follows:

$$\% \ Ferrous \ \ ion \ chelated = \frac{(A_{co} - A_{A(t)}) \times 100}{A_{co}}$$

Where, A_{co} is Absorbance of the control and $A_{(A)(t)}$: is Absorbance of the extracts/standard

Kinetics of Extraction

5 g each of the ground cashew bark weighed into 10 pieces of 100 mL conical flask containing 25 mL of solvent was placed in a water bath shaker. The flasks were removed every 5 minutes interval and filtered. The filtrates were then evaporated to dryness and the amount of the extract was determined gravimetrically. Effect of temperature on the quantity extracted is by repeating the procedure above at 25, 30, 35 and 50 °C considering the stability of the extract with increasing temperature.

RESULTS AND DISCUSSION

Proximate Analysis

The proximate analysis of the stem bark of *A. occidentale* is summarised in Table 1 below. It is observed that the crude fibre is about 27.03; the total carbohydrate content reveals that the plant is rich in lignin. The ash and crude fibre shows that the bark is rich in fibre.

Table 1: Proximate Analysis of stem bark of *A. occidentale*

Parameter	%
Moisture Content	11.15 ± 1.15
Ash Content	2.22 ± 0.29
Crude Fibre Content	27.03 ± 2.45
Carbohydrate	50.31 ± 2.50
Protein content	9.30 ± 3.50

Phytochemical Analysis

The result of the phytochemical analysis is shown in Table 2 below. There are more constituents in the ethanol extract than in water. This is a clear indication of the role of solvent – solute interaction and ability of ethanol molecules to permeate and interact with the components of the stem bark than ordinary water.

Table 2: Phytochemical Constituents of Cashew Stem bark Extract

Constituents	Results	
	Ethanol Extract	Water Extract
Alkaloids	ND	ND
Saponnins	ND	ND
Poly-phenolics and flavonoids	+++	++
Tannins	ND	ND
Terpenoids	+	ND
Steroids	++	+

ND = Not Detected, + = Weakly Detected, ++ = Mildly Detected, +++ = Strongly Detected

Antioxidant Activity of the Extract

The antioxidant activities were investigated with ability of the extract to inhibit the rate of free radical scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide scavenging and ferrous ion chelating activities. Table 3 below shows the effect of the extract on the inhibition processes. The free radical scavenging by DPPH is in the order of ethanol extract > water with IC_{50} values of 3.13 and 2.42 mg/ml inhibition respectively. Ferrous chelating has IC_{50} values of 3.23 and 2.50 mg/ml, while hydroxyl radical scavenging has IC_{50} values of 1.96 and 1.68 mg/ml respectively for the extract obtained from ethanol and water respectively. This may be attributed to the quantity of the antioxidants in the two extracts, since inhibition is dependent on the number of components extracted.

Table 3: Antioxidant Activity of Water and Ethanol Extracts of the Stem bark of *A. occidentale*

Parameters	Ethanol Extract	Water Extract
DPPH Radical Scavenging (IC_{50} mg/ml)	3.13 ± 0.071	2.42 ± 0.051
Chelating ability of Ferrous ion (IC_{50} mg/ml)	3.23 ± 0.031	2.50 ± 0.062
Hydroxyl radical scavenging (IC_{50} mg/ml)	1.95 ± 0.041	1.68 ± 0.034

Kinetics of Extraction

The rate of extract diffusion from the plant matrix into the solvent's medium at different temperatures is important for the designing of a proper extraction process. The quantity extracted as a function time (Q_t), was subjected to pseudo first order and second order kinetic model. The models are according to equations 1 and 2 below, details of which have been explained elsewhere ((Kareem, *et al.*, 2014; Adeogun, *et al.*, 2011; Adeogun, *et al.*, 2012). Table 4 below shows the rate constant k_1 and equilibrium quantity of the extract (Q_o) obtained from the least square fit of the Q_t vs T for the two kinetic models models below.

$$Q_t = Q_o(1 - e^{-k_1 t}) \quad (1)$$

$$Q_t = \frac{Q_o^{\,2} k_2 t}{1 + Q_0 k_2 t} \quad (2)$$

The values of R^2 (i.e. regression analysis which compared the agreement of the model with the experimental data) when compared for the two rate constants showed that the two models can be used to explain the kinetic of antioxidant activity. Therefore further statistical analysis is desirable for correct adaptation of a better model. Hence the percentage error function (the basis of which has also been explained) was used to measure the differences (% SSE) in the calculated and experimental values of equilibrium Q_o. Table 4 below shows that the values of % SSE are lower in pseudo first order model than the second order model for the ethanol extraction process while the reverse is the case for the water extraction process. The data fits better in first order model for ethanol extraction while the second order model best explained the water extraction processes. Figure 1 shows the kinetic fits for the pseudo first and second kinetic model for the two processes.

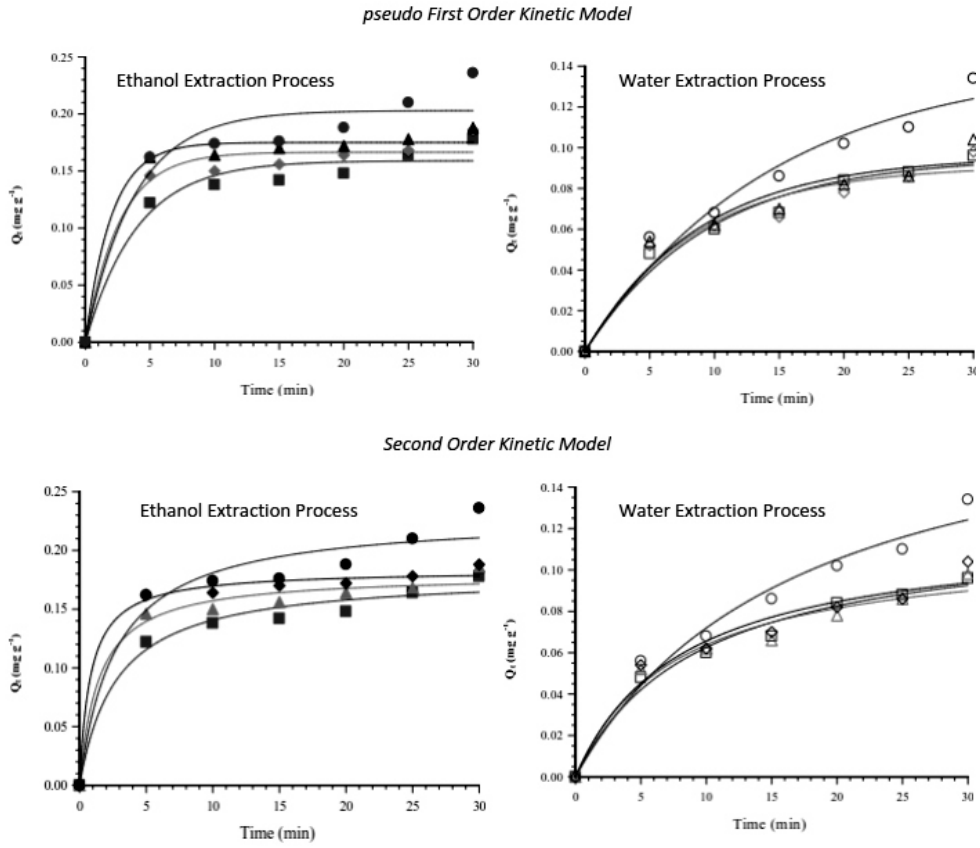

Figure 1: Pseudo first order and second kinetic fit for the extraction process

Extraction Isotherms

Mathematical models proposed for the extraction process are simply empirical equations that fit to experimental data mostly based on mass transfer theory. The power law, Peleg as well as intra particulate diffusion models were further used to quantify and compare the extraction isotherms of antioxidants by water and ethanol from *A. occidentale*.

The power law model is used to reveal the diffusion mechanism of an active substance through non-swelling particles and is described by Sinclair and Peppas (1984) by equation 3 below:

$$Q_t = Bt^n \qquad (3)$$

where Q_t is the amount quantity extracted, B is a constant incorporating the characteristics of the particle-active substance system and n is the diffusion exponent, characterizing the mass transfer mechanism which is usually less than 1. As shown in Table 5, the n values for this study ranges between 0.078 and 0.207 for ethanol extraction while the value is higher in water between the range, 0.37 and 0.52. This could be attributed to mass transfer mechanism being more favoured in ethanol system than in water. It also corroborates the results in Table 2 which shows that components are well detected in ethanol extract. The values of B range in reverse order with the n values and slightly increased with increase in temperature in ethanol but were fairly constant in water which is likely due to the higher volatility of ethanol which exposes the particle-active substance as the temperature is increased. The initial rate of the intra-particle diffusion is given by the following expression:

$$Q_t = k_{id} t^{0.5} + Ci \qquad (4)$$

where k_{id} is the intra-particle diffusion rate constant (mg g^{-1}min $^{0.5}$) and C_i is intercept and a measure of surface thickness. Table 5 shows the parameter for the least square fit for the model. A close look at the fits revealed multi-stage extraction processes in the two solvents. The diffusion rate constant is higher in ethanol than in water while a lower surface thickness is recorded in water.

Peleg's model is actually a hyperbolic model and is of the form:

$$Q_t = \frac{t}{K_1 + K_2 t} \quad (5)$$

where K_1 and K_2 are constants, Q_t is concentration at any time t of extraction. Constant K_1 is related to the extraction rate $B_0 = \frac{1}{K_1}$ at the beginning of the process (t = 0), while constant K_2 is related to maximum extraction yield as $Q_e = \frac{1}{K_2}$ (t $\rightarrow \infty$, extract at equilibrium). A careful look at the values of Q_e obtained shows a similar trend with those obtained from second order fit parameters for the two solvents. The extraction rate at the beginning is higher in ethanol than water hence a quick saturation of the former.

On the average, the best fits are obtained in the two solvents when the Power law and Peleg models were adopted; this is evident from the plot obtained from these fits (Fig. 2). However when the values of their R^2 are compared, they are in the order of Power law model > Peleg Model > Intra-particulate diffusion model. This is also obvious from the plots in Figure 2.

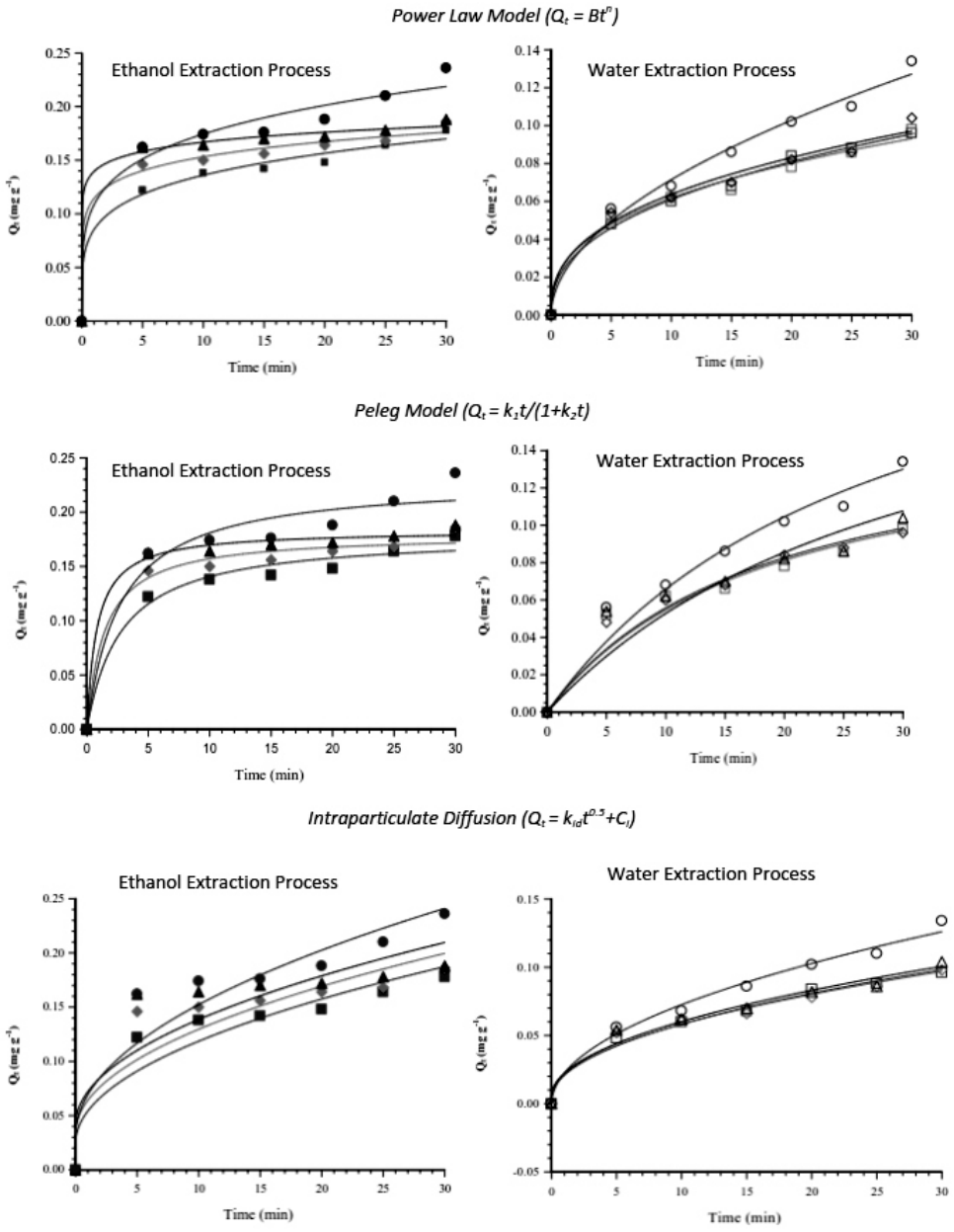

Figure 2: Isothermal fits for the extraction process

Table 4: Comparison of the pseudo first- and second-order extraction rate constants and calculated and experimental Q_o values at different temperature of extraction processes.

	Ethanol									Water							
		First order				Second order				First order				Second order			
Temp K	Q_o (Exp) (mg/g)	Q_o (cal.) (mg/g)	k_1 (min^{-1})	R^2	% SSE	Q_o (cal.) (mg/g)	k_2 ($g(mg\ min)^{-1}$)	R^2	% SSE	Q_o (cal.) (mg/g)	k_1 (min^{-1})	R^2	% SSE	Q_o (cal.)	k_2 ($g(mg\ min)^{-1}$)	R^2	% SSE
298	0.138	0.159	.....	0.994	0.009	0.180	2.007 ± 0.86	0.996	0.017	0.096	0.105 ± 0.03	0.995	0.017	0.122	0.866 ± 0.39	0.997	0.007
303	0.150	0.167	0.379 ± 0.13	0.998	0.007	0.180	3.858 ± 2.09	0.997	0.012	0.091	0.119 ± 0.03	0.99	0.024	0.113	1.151 ± 0.52	0.994	0.015
308	0.164	0.175	0.495 ± 0.21	0.996	0.004	0.183	6.775 ± 5.08	0.999	0.008	0.096	0.114 ± 0.03	0.989	0.028	0.119	1.026 ± 0.45	0.993	0.018
323	0.174	0.203	0.263 ± 0.01	0.989	0.012	0.230	1.613 ± 0.55	0.993	0.023	0.142	0.068 ± 0.02	0.992	0.013	0.195	0.300 ± 0.12	0.994	0.009

Table 5: Isotherm parameters for the extraction of process in Ethanol and water

Models		Power law			Intra-particulate Diffusion			Peleg				
Parameters	Temp K	B ($mg\ g^{-1}min^{-n}$)	n	R^2	k_{id} ($mg\ g^{-1}\ min^{-0.5}$)	C_i ($mg\ g^{-1}$)	R^2	K_1	K_2	B_o ($mg\ g^{-1}min$)	Q_e ($mg\ g^{-1}$)	R^2
Ethanol Extraction Process	298	0.085	0.202	0.998	0.03	0.025	0.983	15.453	5.567	0.065	0.18	0.996
	303	0.114	0.127	0.999	0.03	0.034	0.974	8.026	5.562	0.125	0.18	0.997
	308	0.14	0.078	0.999	0.031	0.042	0.967	4.394	5.454	0.228	0.183	0.999
	323	0.108	0.207	0.997	0.038	0.031	0.982	11.763	4.353	0.085	0.23	0.996
Water Extraction Process	298	0.024	0.408	0.999	0.017	0.004	0.997	121.794	5.933	0.008	0.169	0.991
	303	0.026	0.373	0.997	0.017	0.005	0.995	122.968	5.918	0.008	0.169	0.984
	308	0.026	0.384	0.997	0.017	0.005	0.994	156.391	4.224	0.006	0.237	0.975
	323	0.021	0.525	0.998	0.023	-0.001	0.997	116.106	3.797	0.009	0.263	0.991

Extraction Thermodynamic

Since it is not easy to determine the total concentration of extractable matter in a given sample, a transition state approach was adopted to relate the rate constant to the thermodynamic parameters. Erying equation relates the activation parameter with the rate constant as follows (Kitanovi *et al.*2008; Lafka *et al.*2013; Levenspiel, 2003

$$k = \frac{k_B T}{h} e^{\Delta S^{\#}/R} e^{-\Delta H^{\#}/RT} \quad (6)$$

where k is rate constant, k_B and h are Boltzmann and Planck constants respectively while $\Delta H^{\#}$ and $\Delta S^{\#}$ are activation energy and entropy respectively.

The expression above can be rearranged and linearized as follows;

$$\ln\left(\frac{k}{T}\right) = \left(\ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\#}}{R}\right) - \frac{\Delta H^{\#}}{RT} \quad (7)$$

A plot of $\ln\left(\frac{k}{T}\right)$ vs reciprocal of temperature will give a slope of $\left(\ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{\#}}{R}\right)$ and intercept of $\frac{\Delta H^{\#}}{R}$. The value of $\Delta G^{\#}$ can then be obtained from:

$$\Delta G^{\#} = \Delta H^{\#} - T\Delta S^{\#} \quad (8)$$

Fig. 3 is the least square fit of equation (7) above and Table 6 shows the thermodynamic parameters for the extraction process.

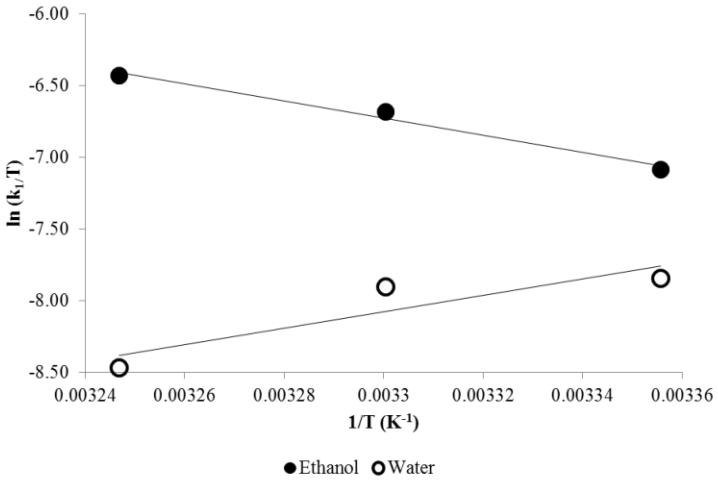

Fig. 3: Thermodynamic fits for the Extraction Process.

$\Delta H^{\#}$ values of 49.67 and 47.37 kJ mol^{-1} obtained for the ethanol and water extraction processes respectively showed that activation of the extraction processes are endothermic. The $\Delta S^{\#}$ values of -0.09 and -0.42 kJ $mol^{-1}K^{-1}$ obtained for the ethanol and water extraction processes respectively suggest higher activation entropy in ethanol than in water extraction processes. The $\Delta G^{\#}$ values and other thermodynamic activation parameters obtained for the two extraction processes are comparable to values obtained in the literature (Topallar and Gecgel, 2000).

Table 6: Thermodynamic parameters for the extraction process

	Ethanol					Water				
Temp	k_1	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (kJ $mol^{-1}K^{1}$)	$\Delta H^{\#}$ (kJ mol^{-1})	R^2	k_1	$\Delta G^{\#}$ (kJ mol^{-1})	$\Delta S^{\#}$ (kJ $mol^{-1}K^{-1}$)	$\Delta H^{\#}$ (kJ mol^{-1})	R^2
298	0.250	76.36	-0.09	49.67	0.985	0.105	78.08	-0.42	47.37	0.813
303	0.379	76.81				0.119	80.19			
308	0.495	77.26				0.114	82.29			
323	0.263	78.60				0.168	88.61			

CONCLUSION

The results of this study show that water and ethanol extracts of the bark of *A. occidentale* contain constituents with antioxidant activities. The results revealed that ethanol extracts have higher constituents than water. Similarly, the antioxidant activity of the ethanol extract is higher than that of water. The extraction processes fitted well with pseudo first order kinetics in ethanol while the water extraction process fitted better with second order kinetics. Both kinetic models gave a higher rate in ethanol than in water. The values of Q_0 i.e. maximum extraction obtainable at equilibrium are also higher in ethanol than in water. The mathematical modelling of the extraction shows a good agreement with Peleg and intra-particulate diffusion model, however, Power law model is best fitted for the two extraction processes. The thermodynamic activation parameters obtained for the two extraction processes are comparable to values obtained in the literatures.

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