Original Research Article

Shelf Life Assessment of *Picralima nitida* and Glibenclamide using Bio-Based Dose-Response Relationship Method

ABSTRACT

Stability testing provides the evidence that ensures the safety, quality, and purity of an active pharmaceutical ingredient or medicinal product. The shelf life of *Picralima nitida* and glibenclamide were evaluated using the bio-based dose-response relationship method via animal model based on their pharmacological activity. Glibenclamide was used as the comparative drug for the assessment of the specifications for *Picralima nitida.* Their shelf life was determined via the accelerated stability studies on the basis of first-order kinetics of degradation and the time required to degrade 10% of a drug at 27°C ($t_{10\%}$). The influence of storage time and temperature on the stability of the drug samples were studied at 45, 60, and 70 °C during the period of one month (1, 2, 3 and 4 weeks). The half-life ($t_{1/2}$) and the toxicity level (LD_{50}) were also estimated. The drug concentrations were found to decline with increase in storage time and temperature. The shelf life of glibenclamide and *Picralima nitida* were found to be 10.54 and 3.15 weeks, respectively. The half-life of glibenclamide and *Picralima nitida* were also established to be 70 and 20.94 weeks, respectively. Their pharmacological activity varied due to the pharmacokinetic profile of the animal models. Also, *Picralima nitida* extract was found to be practically nontoxic on the tested animals. From the study, the use of glibenclamide as a comparative drug aided in the estimation of *Picralima nitida* (herbal drug) capacity to retain its specification (quality and safety) for treatment under the influence of environmental conditions.

Keywords: Accelerated stability study, Degradation kinetics, Glibenclamide, Picralima nitida, Shelf life estimation, bio-based dose-response relationship method, Pharmacological activity, Hypoglycemic effect

1. INTRODUCTION

The purpose of stability testing is to provide evidence on how the quality of an active pharmaceutical ingredient or medicinal product (finished) varies with time under the influence of environmental factors such as temperature, humidity, and light, and the capability to retain its physical, chemical, microbiological, toxicological, protective and informational specifications [1]. Stability studies also assure the identity, potency, and purity of the ingredients, as well as those of the formulated products for regulatory approval [2, 3].

Stability testing involves complex processes because of varieties of influencing factors such as the interaction between the active ingredients and excipients, manufacturing process, type of dosage form, container/closure system used for packaging and light, heat and moisture conditions encountered during shipment, storage and handling. Chemical degradation reactions like oxidation, reduction, hydrolysis or racemization play a vital role in the stability of a pharmaceutical product which depends on conditions like concentration of reactants, pH, radiation, catalysts, as well as the raw materials used and the length of time between manufacture and usage of the product [4]. A pharmaceutical product may undergo physical changes because of impact, vibration, abrasion, and temperature fluctuations such as

freezing, thawing or shearing, which can lead to change in appearance, consistency, content uniformity, clarity (solution), moisture contents, particle size, shape and package integrity thereby affecting its stability. Stability of a pharmaceutical product can also be affected because of microbiological changes like the growth of microorganisms in non-sterile products and changes in preservative efficacy [5].

The finished products of herbal medicine generally have many constituents with low concentration of active constituent(s). Stability testing to ensure safety, quality and purity of herbal drugs is a challenging risk, because the entire herb or herbal product is regarded as the active matter, regardless of whether constituents with defined therapeutic activity are known [6] and the influence of climate, harvesting and biological variance need to be accounted. With regards to the declining efficacy of synthetic drugs, cost, convenience and increasing contradiction, the consumption of natural drugs is relevant owing to their economic significance and ease of access [7, 8].

Picralima nitida extracts have been stated to be effective for the treatment of several ailments [2]. Glibenclamide {5-chloro-N-(4-[N-(cyclohexyl carbamoyl) sulfamoyl] phenethyl)-2-methoxybenzamide} also known as glyburide is an antidiabetic drug [2]. Glibenclamide is amongst the only two oral antidiabetics in the World Health Organization model list of essential medicines [9, 10]. Besides, glibenclamide has been indicated to improve the outcome in animal stroke models by inhibiting brain swelling [11].

In our previous study [2], shelf-life of *Picralima nitida*, glibenclamide, ciprofloxacin, and pefloxacin were determined using the UV spectrometry physicochemical standard technique. The shelf life of ciprofloxacin, pefloxacin, and glibenclamide were obtained as 535.18, 298.17 and 134.31 wks, respectively. Their half-life were also obtained as 3553.85, 1980 and 891.89 wks, respectively. The shelf life and half-life of *Picralima nitida* could not be evaluated using the UV technique because of the existence of complex metabolites in herbal drugs, which can lead to the irregular rise in absorbance value and instability. This lead to the development of a new method that can be applied to determine the shelf-life of *Picralima nitida* (the herbal drug), which was used in this present study. The objective of this study is to evaluate the shelf life of *Picralima nitida* (herbal drug) using the bio-based dose response technique via the animal model, which was also compared to that of an orthodox drug (glibenclamide) at different storage temperatures and time. The half-life, $t_{1/2}$ and acute toxicity (LD_{50}) were also determined.

2. MATERIAL AND METHODS

2.1 Collection and Preparation of the Drug Samples

Picralima nitida (Apocynaceae) seeds were collected from Ihembosi, Anambra state, Nigeria. The pods were washed with clean water and cut to expose the interior part. The seeds were detached from the pulp. The seeds were dried at room temperature for 7 days and the testa was detached manually and dried further. The seeds were crushed. The resulting powder was passed through a 0.25 µm sieve and stored in an airtight container. 200 g of the stored powder was defatted using n-hexane and dried. Methanol of 1 L was added to cover two-thirds of the volume of the container containing the dried defatted seed powder and was allowed to stand for 48 h with occasional shaking. The mixture was filtered and the filtrate was allowed to dry at room temperature. The resinous extract obtained was stored in a refrigerator until further usage to avoid further reaction.

Glibenclamide (hypoglycemic drug) tablets BP 5 mg (diatab®), which was produced in 2009 by Mayer and Baker, Nigeria PLC, Ikeja, Lagos State, Nigeria was acquired from a pharmaceutical store in Awka, Anambra State, Nigeria on February 19, 2012.

2.2 Animal

The animals used in this study consists of 5 weeks old Swiss albino mice (18 – 25 g) and Swiss albino rats (120 – 150 g) which were housed and acclimatized to the environment of the animal house of Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu campus, Nigeria. Prior to the experiments, the animals were abstained from food overnight before measuring their fasting blood glucose level because rodents are nocturnal animals.

2.3 Acute Toxicity Study of *Picralima nitida*

The study was performed using the Lorke method [12] with a total of 16 Swiss albino mice of weight: 18 - 25 g. This was done in two phases but due to the lack of visible signs of acute toxicity and mortality of *Picralima nitida* seed extract, the study was then extended to 3 phases. In the first phase, 3 groups of 3 mice each were orally administered 10, 100 and 1000 mg/kg of the seed extract and signs and symptoms of toxicity and mortality were observed for a period of 24 h and weighed daily for 14 days. For the second phase, 3 mice were distributed into 3 groups of an animal each which were orally administered 1600, 2900 and 5000 mg/kg of the seed extract and observed for another 24 h and weighed daily for 14 days.. Finally, for the third phase, 4 mice were distributed into 4 groups of an animal each given extremely high doses

of 10000, 14000, 16000 and 20000 mg/kg of the seed extract followed by close observation over the period of 24h and weighed daily for 14 days.

2.4 Bioassay with Animal Model

A standard plots of percentage glycemic change against Log dose for glibenclamide and *Picralima nitida* were achieved by dividing the rats into groups of stock concentration of the drugs (glibenclamide 2.5 mg/mL in water of doses: 1, 2, 3, 4 mg/kg and *Picralima nitida* extract 2.5 mg/mL in water of doses: 25, 50, 75, 100, 125, 250 mg/kg). Each group contains 5 rats which were dosed individually according to their calculated weights. Blood samples were withdrawn from their tails and normal glucose level measured with the aid of glucometer (mL/dL) before and after 2 h of drug administration. The mean glycemic change and percentage glycemic change of each group was calculated and tabulated using Eq. 1:

$$\%\ \text{Percentage glycemic change} = \frac{\text{Blood glucose level after 2 hr of oral admin.(mean)} - \text{Normal blood glucose level (mean)}}{\text{Normal blood glucose level (mean)}} \times 100 \qquad (1)$$

The stock solutions of the drugs were then subjected to different temperatures (45, 60, and 70 °C) and the samples were collected at one-week interval for one month to obtain the percentage glycemic change in which the Log doses were extrapolated from the regression equations of their standard plots and the corresponding final concentrations estimated with the Eq. 2:

$$\frac{C_0}{D_0} = \frac{C_1}{D_1} \qquad (2)$$

Where C_0 = Initial concentration (mg/mL), C_1 = final concentration (mg/mL), D_0 = Initial dose (mg/kg), and D_1 = final dose (mg/kg).

All experiments were carried out in the year 2012.

3. RESULTS AND DISCUSSION

3.1 Estimation of *Picralima nitida* Lethal Dose for 50% (LD_{50})

After close observation via the post administration of the seed extract, there was no visible sign of toxicity and weight loss but mortality occurred at the doses of 16000 and 20000 mg/kg. Alkaloids, saponins and their derivatives may contribute to the mortality, which was documented to have a hepatotoxic effect [13 – 15]. A study by Ilodigwe et al. [16] on sub-chronic toxicological studies on *Picralima nitida* leaf extract dictated hepatocellular injury that leads to intracellular enzymes permeability into the bloodstream, which is accompanied by elevated serum Aspartate aminotransferase (AST) and Alanine aminotransaminase (ALT). Also, cholestasis was dictated due to biliary obstruction or hepatic infiltration accompanied by elevated serum Alkaline phosphatase (ALP).

The LD_{50} was calculated as follows [12]:

$$\text{LD}_{50} = \sqrt{(D_0 \times D_{100})} \qquad (3)$$

Where D_0 is the highest dose that gave no mortality (14000 mg/kg), and D_{100} is the lowest dose that produced mortality (16000 mg/kg).

The lethal dose (LD_{50}) for the *Picralima nitida* extract was estimated to be 14967 mg/kg ≈ 14.97 g/kg. This implies that the extract is practically nontoxic on the test animals [17].

3.2 Bioassay Standard Plots of Glibenclamide and *Picralima nitida*

The percentage glycemic change against Log dose (standard plots) of glibenclamide and *Picralima nitida* are presented in Figs. 1-2. The regression equations obtained were used to calculate the final drug concentrations after the accelerated stability studies at different storage conditions (times and temperatures).

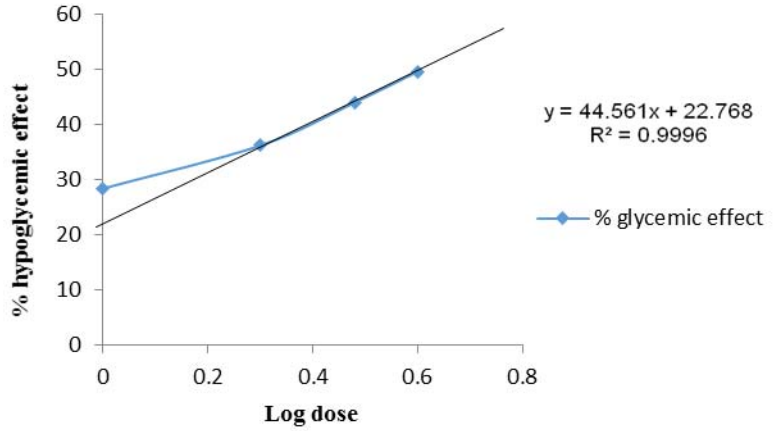

Fig. 1. Bioassay with animal model standard plot of glibenclamide.

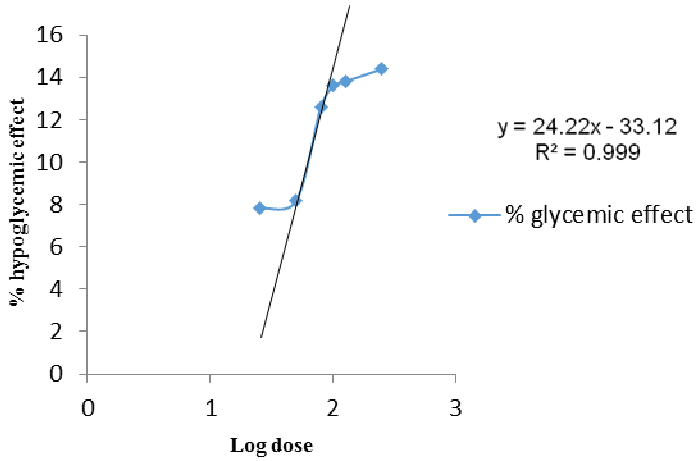

Fig. 2. Bioassay with animal model standard plot of *Picralima nitida.*

3.3 Effect of Storage Time and Temperature on Drug Concentration

The accelerated stability experiment was performed by stressfully conditioning the drug for a period of time (0, 1, 2, 3 and 4 weeks) at high temperatures (45, 60, and 70 °C) using an initial drug concentration of 2.5 and 100 mg/mL for glibenclamide and *Picralima nitida,* respectively. The residual concentrations of glibenclamide and *Picralima nitida* at the different storage times and temperatures were determined as shown in Figs. 3-4 (The plot of concentration of the drug substances remaining against time). It can be observed that the concentrations of glibenclamide and *Picralima nitida* decreased with increase in storage time. Increase in the storage temperature improved the decomposition of glibenclamide, and *Picralima nitida* owing to the fact that molecules are likely to move more rapidly with intensified kinetic energy [18]. This entails that the storage temperature and time have a great impact on the drugs' concentrations.

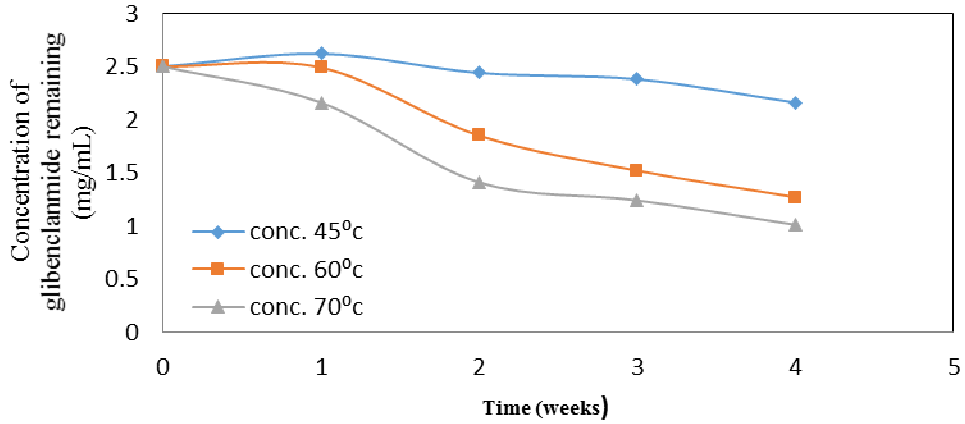

Fig. 3. Effect of storage time and temperature on glibenclamide concentration using animal model.

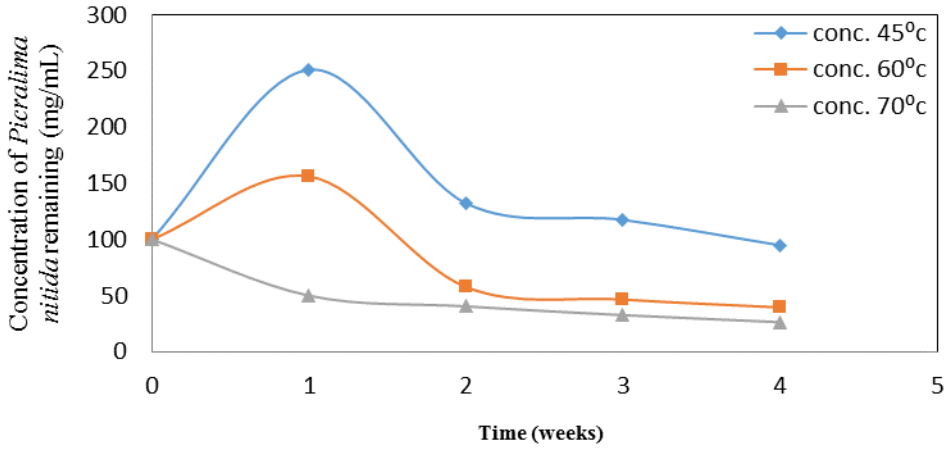

Fig. 4. Effect of storage time and temperature on *Pricralima nitida* concentration using animal model.

3.4 Degradation Kinetics

The accelerated degradation kinetics experiment was performed at elevated temperatures of 45, 60, and 70°C. The degradation kinetic constant (k) was calculated using the first-order rate equation stated as follows [2]:

$$Ln\ C = Ln\ C_0 - k_1 t \quad (3)$$

Where C is the residual drug concentration at time, t; C_0 is the initial drug concentration at time (t = 0); k_1 is the degradation rate constant.

The degradation rate constant, k_1 were calculated (Table 1) from the slopes of the linear plot of Ln conc. and time (Figs. 5-6). The average k_1 can be calculated each week at temperatures of 45, 60 and 70°C. The correlation coefficients (Figs. 5-6) indicates that the degradation data of glibenclamide and *Picralima nitida* fit or didn't fit into the first-order model at all studied temperatures.

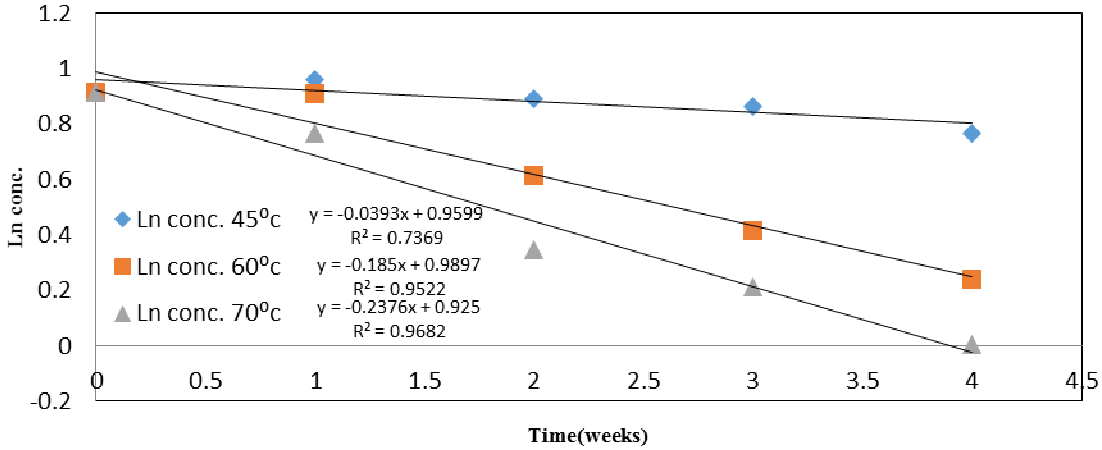

Fig. 5. Degradation kinetics of glibenclamide using animal model.

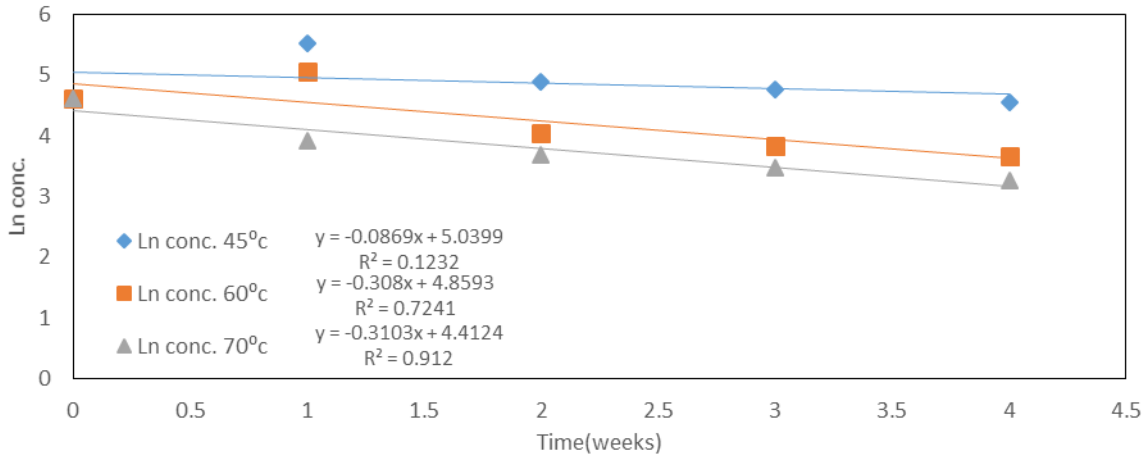

Fig. 6. Degradation kinetics of *Pricralima nitida* using animal model.

3.5 Determination of Shelf-life of the Drug Substances

The theory of accelerated stability analysis is centered on the Arrhenius equation [2]. The influence of temperature on the degradation kinetics was also studied using the Arrhenius equation. The Arrhenius equation gives a correlation between temperature and rate of reaction [2]. The value of the degradation rate constant, k at 27^{0}C (k_{27}) was deduced from the Arrhenius plot (Figs. 7-8) (Ln k values against 1/T) using Eq. 4 [19, 20]:

$$\mathrm{Ln\,k} = \mathrm{Ln\,A} - \frac{E_a}{RT} \quad (4)$$

Where A is the frequency of molecular collisions occurring between the molecules or Arrhenius factor, E_a is the energy of activation (kJ/mol.K), T is the absolute temperature (K) and R is the ideal gas constant (8.314 J/mol.K).

The rate of degradation can also be determined by the energy of activation of a chemical reaction [2, 21]. The activation energy was calculated from the slope of the Arrhenius plots (Figs. 7-8) using Eq. 5:

$$\mathrm{Slope} = \frac{E_a}{R} \quad (5)$$

The activation energies, E_a for the degradation of glibenclamide and *Picralima nitida* (Table 1) were found to be high (since E_a > 50 kJ/mol.K). The higher the activation energies, E_a, the lesser the influence of temperature on the degradation reaction. Based on the result, temperature contributed to the drugs' degradation [22] and also, the pharmacokinetic profile of the animal model also contributed to its degradation.

The shelf-life of glibenclamide and *Picralima nitida* were estimated by substituting the values of k_{27} obtained using Eq. 6 [2]:

$$t_{10\%} = \frac{\mathrm{Ln}\,1.111}{k_{27}} = \frac{0.105}{k_{27}} \quad (6)$$

Where $t_{10\%}$ is the time required for 10% degradation of the drug (the shelf life) and k_{27} is the degradation rate constant at 27°C. The shelf life of glibenclamide and *Picralima nitida* were found to be 10.54 and 3.15 weeks, respectively (Table 1).

Another parameter of importance to drug stability is the half-life ($t_{1/2}$), which is the period of time required for the concentration of drug to be reduced by one-half of the original drug concentration. The half-life of the substances were estimated at 27°C (Table 1) as follows:

$$t_{1/2} = \frac{\mathrm{Ln}\,2}{k_{27}} = \frac{0.693}{k_{27}} \quad (7)$$

The half-life of glibenclamide and *Picralima nitida* were found to be 70 and 20.94 weeks, respectively.

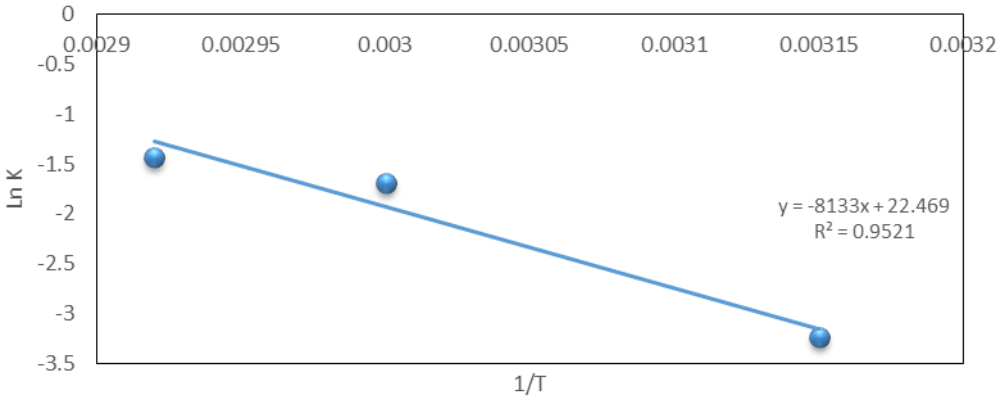

Fig. 7. Arrhenius plot for glibenclamide using animal model.

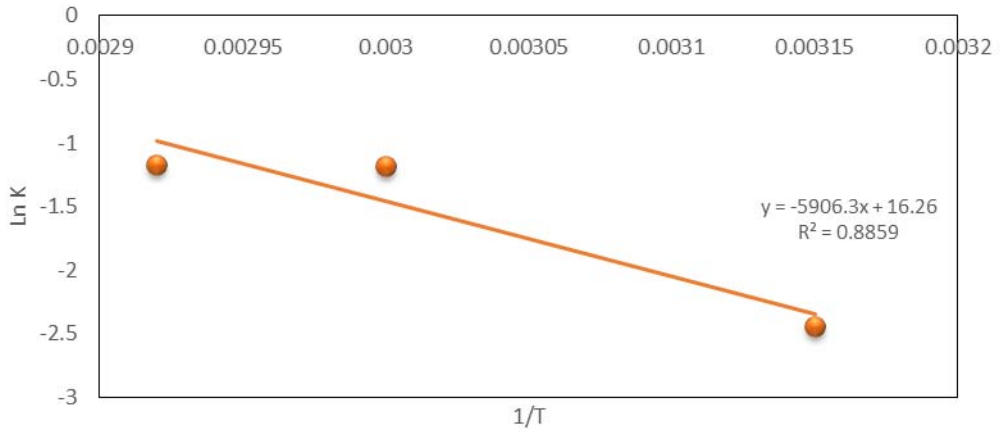

Fig. 8. Arrhenius plot for *Picralima nitida* using animal model.

Table 1: Degradation rate constants calculated at different temperatures, shelf life and half-life of glibenclamide and *Picralima nitida*.

Drug substance	Temp. (°C)	K_1 (week-1)	Absolute Temp. (K)	1/T	Ln K	Arrhenius factor (A)	Activation energy, E_a (kJ/mol.K)	Shelf-life at 27°C (weeks)	Half-life at 27°C (weeks)	Q_{10}
Glibenclamide	27	0.0099	300	0.00333	-4.61389					
	45	0.0394	318	0.00315	-3.23399					
	60	0.185	333	0.003	-1.6874	5.73×10^9	67.62	10.54	70	2.33
	70	0.2376	343	0.00292	-1.43717					
Picralima nitida	27	0.0331	300	0.00333	-3.408					
	45	0.0869	318	0.00315	-2.443					
	60	0.308	333	0.003	-1.17766	1.15×10^9	49.11	3.15	20.94	1.85
	70	0.3103	343	0.00292	-1.17022					

3.6 Q_{10} Method for Shelf-Life Estimation

Simonelli and Dresback's Q_{10} (the factor by which rate constant increases for a 10°C temperature increase) method for shelf-life determination [23] was also applied in this study. It is the ratio between two reaction rate constants. The commonly used Q_{10} values of 2, 3 and 4 relate to the activation energy of reaction at room temperature.

$$Q_{10} = \frac{K_{(T+10)}}{K_T} \quad (8)$$

$$Q_{10} = \exp\left[\frac{-E_a}{R}\left(\frac{1}{(T+10)} - \frac{1}{T}\right)\right] \quad (9)$$

Q_{10} = 4, provides the higher estimate for the increase in rate with increasing temperature and estimate the maximum likely decrease in shelf life with increasing temperature; Q_{10} = 2, provides the lower estimate for the decrease in rate with decreasing temperature and provide the most conservative estimate of the increase in shelf life with decreasing temperature; Q_{10} = 3 gives our most likely estimate.

Q_{10} is applied in the prediction and estimation of the expiration date or shelf-life of drug materials at varying storage temperatures (Eq. 10 and Table 2) and is independent of the reaction order.

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{(T_2-T_1)/10}} \quad (10)$$

Where t_{90} (T_2) is the estimated shelf life, t_{90} (T_1) is the shelf-life at a given temperature. T_1 and T_2 are the varying temperatures.

Table 2: The estimated shelf-life using the Q_{10} method at room temperature.

Drug substance	Q_{10}	Shelf-life at 27°C (weeks)	Shelf-life at 4°C (weeks)	Shelf-life at 37°C (weeks)
Glibenclamide	2.33	10.54	73.75	4.52
Picralima nitida	1.85	3.15	12.97	1.7

4. CONCLUSION

The shelf life of *Picralima nitida* (herbal drug) and glibenclamide (orthodox drug) have been investigated. The stability/shelf life study was done using the bio-based dose respond relationship technique. The shelf life, $t_{10\%}$ (the time required to degrade 10% of a drug at 27°C) was determined by accelerated stability studies. The stability studies were done at temperatures of 45, 60, and 70 °C at 1, 2, 3 and 4 weeks. The first-order degradation kinetics constant (k), half-life ($t_{1/2}$), and toxicity level (LD_{50}) were also evaluated. Storage temperature and time were found to have a great change in the concentration of the drugs. The shelf life of glibenclamide was obtained as 10.54 and that of *Picralima nitida* was found to be 3.15 weeks. The use of glibenclamide (orthodox drug) as a comparative drug assisted in the estimation of *Picralima nitida* (herbal drug) capacity to retain its specification (quality and safety) for treatment under the influence of environmental conditions. From the study, the shelf life of glibenclamide and *Picralima nitida* can be evaluated based on their pharmacological activity using the bio-based dose respond relationship technique but varied due to their pharmacokinetic profile of the animal models.

ETHICAL APPROVAL

All experiments have been reviewed and approved by the appropriate ethics committee of COOUTH Amaku, P.M.B., 5022 Awka, Anambra State, Nigeria with reference number COOUTH/AA/VOL.I.031.

Consent: NA

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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