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Determination of Shelf Life of *Picralima nitida*, Ciprofloxacin and Pefloxacin Using Bio-Based Concentration-activity Relationship Technique

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: The shelf-life of *Picralima nitida* (herbal drug) and two orthodox drugs (ciprofloxacin, and pefloxacin) has been examined.
Methodology: The stability studies were carried out using the bio-based concentration-activity relationship technique. Accelerated stability studies were applied on the basis of first-order degradation kinetics to determine the shelf-life of the drugs at different temperatures (45 – 70°C) and storage times (1, 2, 3 and 4 wks). Ciprofloxacin and pefloxacin were used as the comparative drugs for the estimation of the specifications for *Picralima nitida*. Their half-life ($t_{1/2}$) and temperature coefficient (Q_{10}) were also investigated.
Results: All the drugs proved to be broad spectrum antibiotics and their concentrations were found to decrease with increase in storage time and temperature. Ciprofloxacin proved to be more active and stable than pefloxacin followed by *Picralima nitida,* but lost its activities against the organisms at the stressed condition, respectively. *Picralima nitida* retained its activity more at stressed condition because of the presence of active metabolites. The shelf-life (including the half-

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life) of ciprofloxacin was found to be 80.31 wks (533.08 wks) against *Bacillus subtilis*, pefloxacin was found to be 43.5 wks (288.75 wks) against *Bacillus subtilis* and 0.25 wks (1.65 wks) against *Samonella typhi*; *Picralima nitida* found to be 5.83 wks (38.72 wks) against *Bacillus subtilis*, 0.41 wks (2.69 wks) against *Samonella typhi* and 0.52 wks (3.45 wks) against *Pseudomonas aureginosa*.

Conclusion: The shelf-life of *Picralima nitida*, ciprofloxacin, and pefloxacin were successfully determined using the bio-based concentration-activity relationship technique; ciprofloxacin and pefloxacin were also successfully used as the comparative drugs for the estimation of the specifications for *Picralima nitida* in treatment based on their inhibitory activity but varies with sensitivity activities on different bacteria (or micro-organisms).

Keywords: Accelerated stability study; ciprofloxacin; degradation kinetics; pefloxacin; picralima nitida; shelf life determination; bio-based concentration-activity relationship technique.

1. INTRODUCTION

During the development of a drug, pharmaceutical analysis, and stability studies are necessary to determine and guarantee the identity, potency, and transparency of itsactive ingredients, along with their finished products [1,2]. Drug stability studies are generally carried to establish the degradation of its active ingredient [3,4]. The stability deviation of drug substance or product based on its manufacturing, packaging, storage, distribution, or in-use significantly affects the specification of drug product for use by the patient; it's a matter of apprehension for health regulatory authorities and pharmaceutical industries [5,6]. Stability is defined as the extent of pharmaceutical drug material to maintain the specifications of its active ingredient at a period of time to ensure its quality and safety [7,8].

Stability testing of drug materials provides information on the drug variation with time under the impact of different environmental conditions including temperature, humidity and light [9,10]. The stability of a drug product gives additional information on the potential storage conditions in the course of raw materials processing, the intermediates, final products and the shelf-life/expiration date of the pharmaceutical substance [7]. Shelf-life is usually estimated from real-time stability tests and accelerated stability tests [11,12]. In real-time stability testing, close observation of a product stored at recommended storage conditions is required until it fails the stated specification. In accelerated stability tests, a product is stored at elevated stress conditions such as temperature, humidity, and pH [12]. Degradation at the acclaimed storage conditions can then be predicted via known relationships between the acceleration factor and the rate of degradation.

Herbal drugs used for decades play an inevitable role in drug discovery and development, treatment of diseases and revitalization of body systems [7,13]. 80% of the world populates rely on herbal medicine [14,15]. The use of herbal medicines and phytonutrients or nutraceuticals continue to expand rapidly across the world with many people now resorting to these products for the treatment of various ailments in different national healthcare settings [14,16]. Besides, the declining efficacy of synthetic drugs, cost, convenience and increasing contradiction of their use makes the consumption of natural drugs relevant [17,18]. Data on the quality, safety, and efficacy of herbal medicine is limited notwithstanding its economic significance and ease of access; therefore, the need for the examination of their safety to improve the modern day consumer's reliance.

Picralima nitida extracts have been reported to be effective for hypertension and gastrointestinal disorders [17]; it's used as a cough suppressant [19], treatment of viral infections [20] and hypoglycaemic agent in the treatment of diabetes [19]. It also possesses antibacterial effect [21,22], anti-inflammatory and antipyretic activity [23], analgesic activity [24,25], and anti-parasitic effect [26,27]. Ciprofloxacin {1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3 quinolinecarboxylic acid} and pefloxacin {1-ethyl-6-fluoro-7-(4- methyl piperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid} are synthetic broad-spectrum antibiotic active against both gram-positive and gram-negative bacteria [28]. Pefloxacin, an analog of norfloxacin is a synthetic chemotherapeutic agent used to treat severe and life-threatening bacterial infections [29].

In our previous study [7], the shelf life of *Picralima nitida*, Glibenclamide, Ciprofloxacin and Pefloxacin were determined using UV

spectrometry physicochemical method. 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 this technique due to the presence of complex metabolites in the herbal drug, which lead to in the irregular increase in absorbance and instability. The purpose of this study is to use the bio-based concentration-activity relationship approach to evaluate the shelf life of *Picralima nitida* (herbal drug) at different storage temperatures and times which was also compared to the shelf-life of orthodox drugs (ciprofloxacin and pefloxacin).

2. MATERIALS AND METHODS

2.1 Collection and Preparation of the Drug Samples Subheading

Picralima nitida (Apocynaceae) seeds were collected from Ihembosi, Anambra state, Nigeria. The pods were rinsed with clean water and cut longitudinally to expose the interior part. The seeds were separated from the pulp. The seeds were dried at room temperature for 7 days and the testa was removed manually and dried further. The seeds were pulverized. 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 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 usage to avoid further reactions.

The orthodox drugs used in the study were:

(1). Ciprofloxacin hydrochloride (antibacterial drug) tablets USP 500 mg (gecip®) manufactured in 2010 by MICRO lab limited, Bangalore, India and marketed by Geneith Pharm LTD, Oshodi, Lagos State, Nigeria.

(2). Pefloxacin (antibacterial drug) tablets 400 mg (peflomed®) manufactured in 2011 by Bharat Parenterals LTD, Barode Gujarat, India and marketed by Evans medical PLC, Agbara, Ogun State, Nigeria.

All drugs (tablets) were obtained from a pharmaceutical store in Awka, Anambra State, Nigeria on February 19, 2012.

2.2 Phytochemical Analysis of *Picralima nitida*

Using the standard methods described by Trease and Evans [30], the *Picralima nitida* seed powder was analyzed for the presence of secondary metabolites.

A. Analysis for alkaloids: 2 g of the seed powder was introduced into a test tube containing 20 mL of 5% sulfuric acid in 50% ethanol which was heated on a water bath for 10 min, allowed to cool and filtered. The filtrate was placed in a 100 ml separating funnel and alkalinized with ammonia solution in which the aqueous alkaline solution was separated and extracted with two portions of 5 mL dilute sulfuric acid. The extract was treated with few drops of Mayer's, Wagner's and Dragendoff's reagent which gives milky, reddish brown and brick red precipitates, respectively indicating the presence of alkaloids.

B. Analysis for flavonoids: 0.2 g of the seed powder was introduced into a test tube containing 10 mL of ethyl acetate which was heated on a water bath for 3 min, allowed to cool and filtered. 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. The layers were allowed to separate and the yellow color observed in the ammonical layer indicates the presence of flavonoids.

C. Analysis for saponins: 0.25 g of the seed powder was introduced into 100 mL beaker containing 20 mL of distilled water which was gently boiled on a water bath for 2 min, allowed to cool and filtered. 5 mL of the filtrate was diluted with 20 mL of distilled water and shook vigorously. Stable froth (foam) upon standing indicates the presence of saponins. Also, 5 mL of Fehling's solution (equal parts of I and II) was added to 10 mL of the filtrate and heated. A reddish precipitate observed indicates the presence of saponins.

D. Analysis for tannins: 1 g of the seed powder was introduced into 100 mL beaker containing 50 mL of distilled water which was gently boiled on a water bath, allowed to cool and filtered. Few drops of ferric chloride were added to 3 mL of the filtrate. A greenish black precipitate observed indicates the presence of tannins.

E. Analysis for glycosides: 0.5 g of the seed powder was introduced into a test tube containing 5 mL of dilute sulfuric acid which was boiled in a water bath for 15 min, allowed to cool, filtered and neutralized with 20% potassium hydroxide solution. 5 mL of Fehling's solution (equal parts of I and II) was added to 10 mL of the filtrate and heated. A more dense brick red precipitate observed indicates the presence of glycosides.

F. Analysis for steroids and terpenoids: 9 mL of ethanol was added to 1 g of the seed powder, refluxed for few minutes and filtered. The filtrate was concentrated to 2.5 mL on a boiling water bath and 5 mL of hot water added. The mixture was allowed to stand for 1 h to remove waxy matter by filtration and the filtrate extracted with 2.5 mL of chloroform using separating funnel. To 0.5 ml of chloroform extract, 1 mL of concentrated sulfuric acid was carefully added to form a lower layer with a reddish brown interface which indicates the presence of steroids. Also, 0.5 mL of chloroform extract was evaporated to dryness on a water bath and 3 mL of concentrated sulfuric acid was carefully added and heated for 10 min on a water bath. A grey color observed indicates the presence of terpenoids.

G. Analysis for resins: 0.2 g of the seed powder was extracted with 15 mL of 96% ethanol. The alcohol extract was introduced into 100 ml beaker containing 20 mL of distilled water. A precipitate observed indicates the presence of resins.

H. Analysis for carbohydrates: 0.1 g of the seed powder was introduced into a test tube containing 5 mL of distilled water, shook vigorously and filtered. 2 mL of Benedict's reagent was added to 1 mL of the filtrate, shaken and heated on a water bath for 5 min. A rusty brown precipitate observed indicates the presence of reducing sugar. Also using Molisch's test, 0.1 g of the seed powder was introduced into a test tube containing 2 mL of distilled water, boiled and filtered. Few drops of Naphthalol solution in ethanol were added to the filtrate and concentrated sulfuric acid gently poured down the side of the test tube to form a lower layer. A purple interfacial ring observed indicates the presence of carbohydrates.

I. Analysis for proteins: 1 g of the seed powder was introduced into 100 mL beaker containing 50 mL of distilled water which was gently boiled on a water bath, allowed to cool and filtered. Two drops of Million's reagent was added to 3 mL of the filtrate. A white precipitate observed indicates the presence of proteins.

J. Analysis for fats and oil: 0.1 g of the seed powder was pressed in-between filter papers. The translucency of the filter papers indicates the presence of fats and oil.

K. Analysis for phenols: 0.5 g of the seed powder was treated with lead acetate solution. The precipitate observed indicates the presence of phenols.

2.3 Stability Study Using Bacteria Assay

Standard plots of IZD^2 (inhibition zone diameter) against *Picralima nitida*, ciprofloxacin and pefloxacin concentrations were determined by preparing agar diffusion (0.1 mL of broth cultures of bacteria mixed with molten nutrient agar and poured into the petri dish which was rotated gently to ensure even distribution and allowed to set), which was divided into six parts and holes bored with 9 mm cork borer. Two-fold serial dilutions were made into six different concentrations of the stock solutions (ciprofloxacin hydrochloride-25 mg/mL in distilled water, pefloxacin-100 mg/mL in distilled water and *Picralima nitida* seed extract-200 mg/mL in $DMSO_4$) were prepared and 2 drops of each drug concentration introduced into the holes with a pipette. The agar diffusion was allowed to stand for 15 min at room temperature of 27°C before incubation for 24 h at 37°C. The developed IZD^2 were measured and the diameter of the cork bearer subtracted. All reagents used in the study were of analytical grade.

The stock solutions of the drugs were then subjected to different temperatures (45°C, 60°C and 70°C) and the samples collected at a one-week interval for one month (1, 2, 3 and 4 wks) to obtain their IZD^2 from agar diffusion in which their corresponding final concentrations were evaluated from the regression equations of their standard plots.

All experiments were carried out from June - August 2012.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis of *Picralima nitida*

From the phytochemical analysis, *Picralima nitida* was found to possess many secondary

metabolites with several activities experimentally observed like cough suppressant anodyne, anesthetic and aphrodisiac [31], antibacterial [21,22], antiparasitic and antileishmanial [26,27,32-34], antiviral [35], anti-inflammatory and antipyretic [23,25], analgesic [24], hypoglycemic [31,36-39], hypotensive [40], hypocholesterolemic, anticarcinogenic, antioxidant, antimutagenic and the ability to modify gene expression [41].

3.2 Standard Plots of Ciprofloxacin, Pefloxacin, and *Picralima nitida*

Figs. 1-3 show the standard plots (IZD^2 against concentration) of ciprofloxacin, pefloxacin and *Picralima nitida* at different dilution concentrations with their respective IZD^2 against different bacteria. The regression equations obtained from the standard plots were used further to calculate the final concentrations of the drug samples after the accelerated stability studies under the stressed condition of different time intervals and temperatures.

3.3 Effect of Storage Time and Temperature on Drug Concentration

With reference to the accelerated stability study, the drugs were stressfully conditioned to high or elevated temperatures over a period of time. The residual concentration of ciprofloxacin, pefloxacin, and *Picralima nitida* after storage times of 0, 1, 2, 3 and 4 wks at different storage temperatures of 45, 60 and 70°C were evaluated using the initial concentration of 25, 100 and 200 mg/ml for ciprofloxacin, pefloxacin and *Picralima nitida*, respectively. The final concentration of the drug substances was plotted against time (Figs. 4-9), which showed decreased ciprofloxacin, pefloxacin, and *Picralima nitida* concentrations with increase in storage. The decomposition of ciprofloxacin, pefloxacin and *Picralima nitida* was improved with increasing temperature since the molecules tend to move faster with amplified kinetic energy [42].This implies that the storage time and temperature had a great influence on the concentration of the drug substances.

3.4 Kinetics of Degradation

Using elevated temperatures of 45, 60 and 70°C, the accelerated degradation kinetics was studied. First-order rate equation was used to evaluate the degradation kinetic constant, k_1 [7]:

$$LnC = LnC_0 - k_1 t \quad (1)$$

Where C and C_0 is the residual concentration of the drug at time, t and drug concentration at time (t=0), respectively. k_1 is the degradation rate constant, which can be evaluated from the slopes of the linear plots of LnC versus t (Figs.10-15).The average k_1 can be calculated each week at temperatures of 45, 60and 70°C.

The degradation rate constant, k_1 obtained at various temperatures for the drug substances are presented in Table 1. The correlation coefficients (R^2<0.95) show that the degradation experimental data of ciprofloxacin, pefloxacin, and *Picralima nitida* didn't fit or partially fit into the first-order model at all studied temperatures.

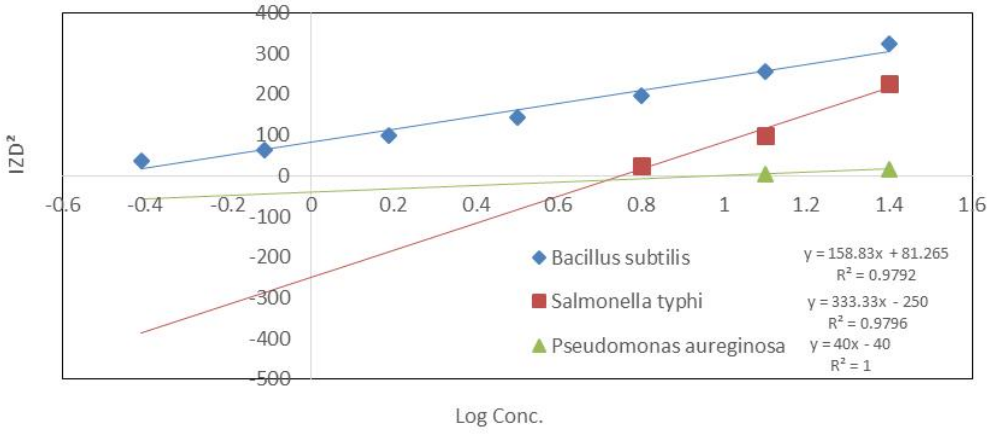

Fig. 1. Standard plot of ciprofloxacin with *Bacillus subtilis, Salmonella typhi,* and *Pseudomonas aureginosa*

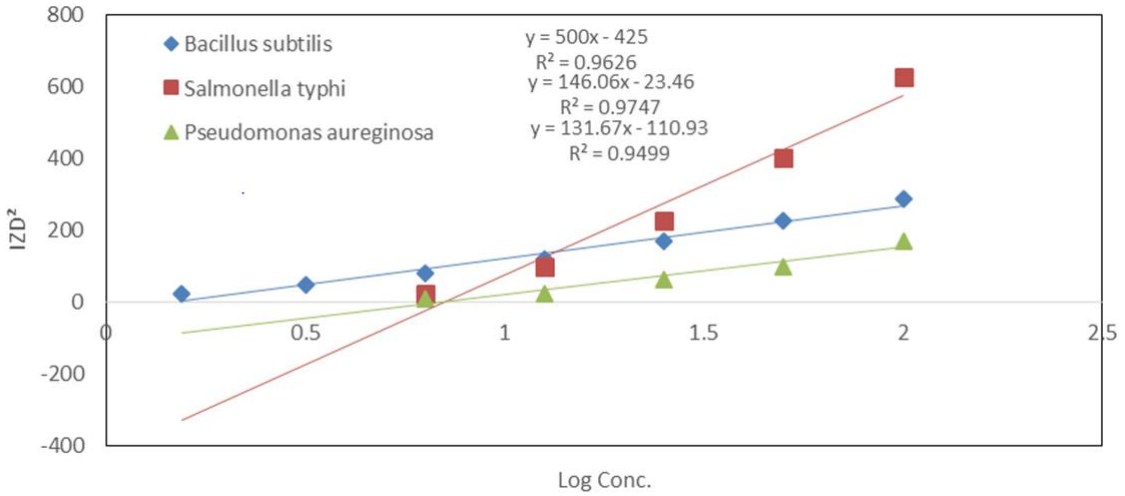

Fig. 2. Standard plot of pefloxacin with *Bacillus subtilis, Salmonella typhi,* and *Pseudomonas aureginosa*

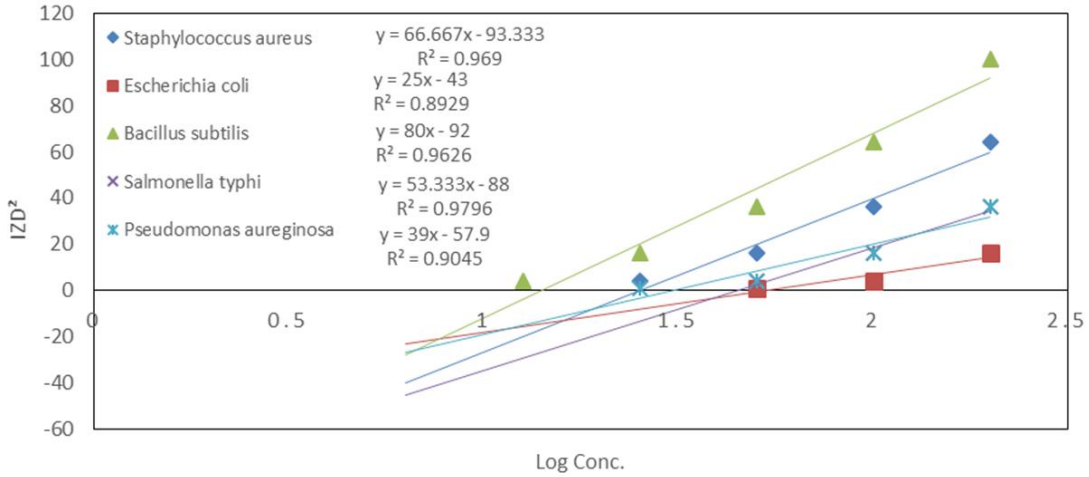

Fig. 3. Standard plot of *Picralima nitida* with *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Salmonella typhi,* and *Pseudomonas aureginosa*

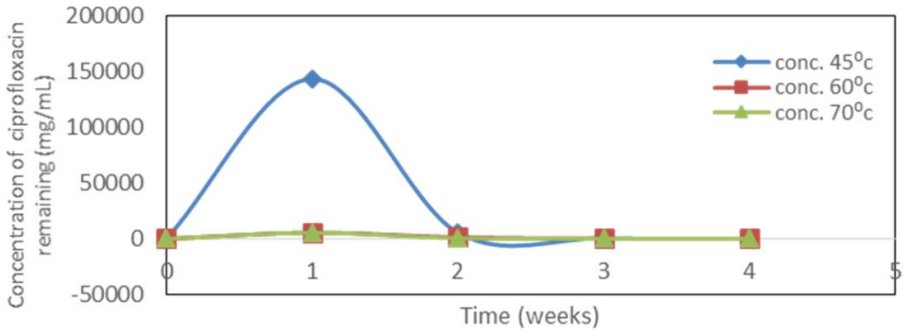

Fig. 4. Effect of storage time and temperature on ciprofloxacin concentration with *Bacillus subtilis*

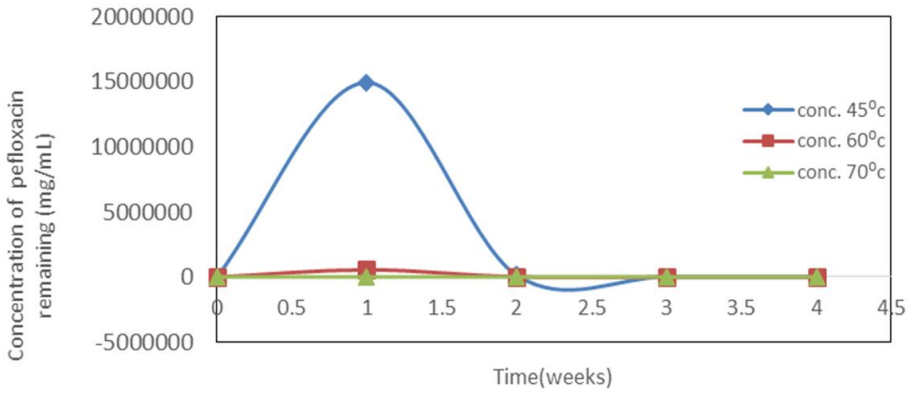

Fig. 5. Effect of storage time and temperature on pefloxacin concentration with *Bacillus subtilis*

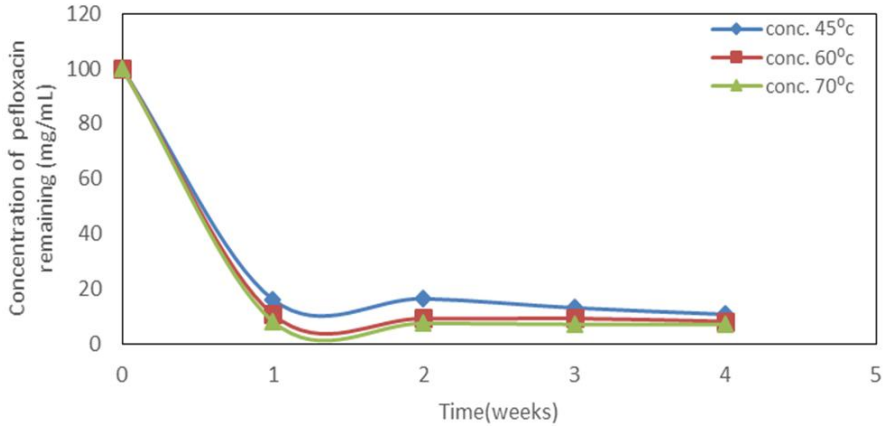

Fig. 6. Effect of storage time and temperature on pefloxacin concentration with *Samonella typhi*

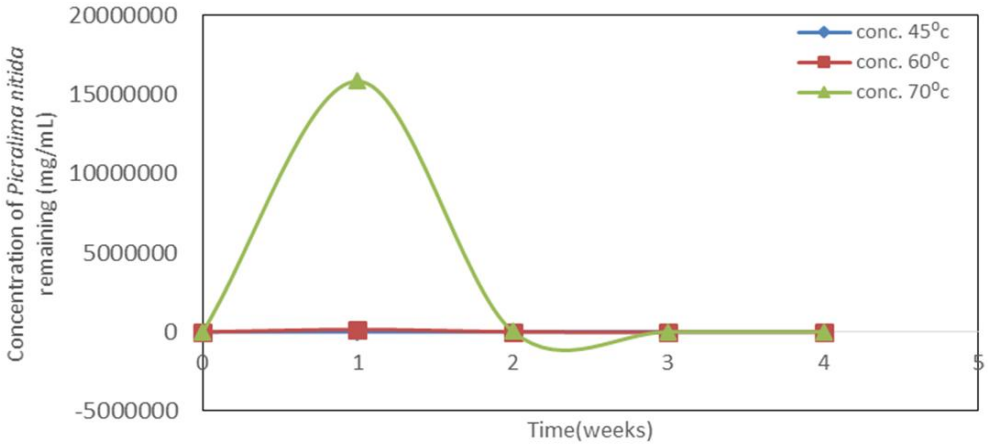

Fig. 7. Effect of storage time and temperature on *Pricralima nitida* concentration with *Bacillus subtilis*

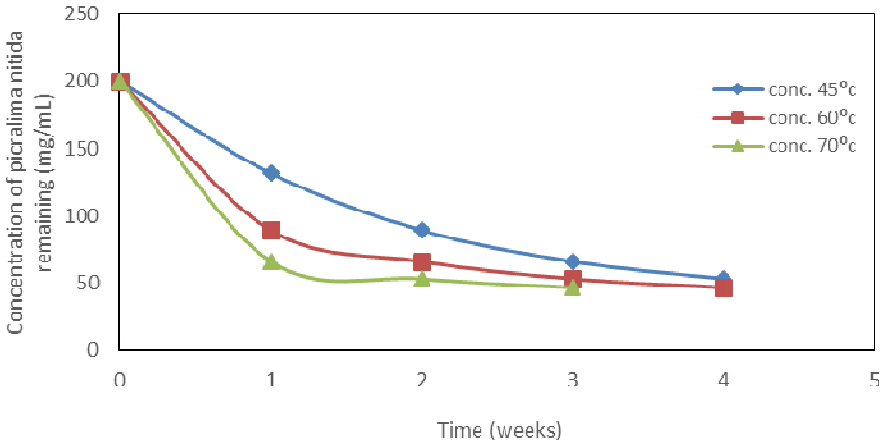

Fig. 8. Effect of storage time and temperature on *Pricralima nitida* concentration with *Samonella typhi*

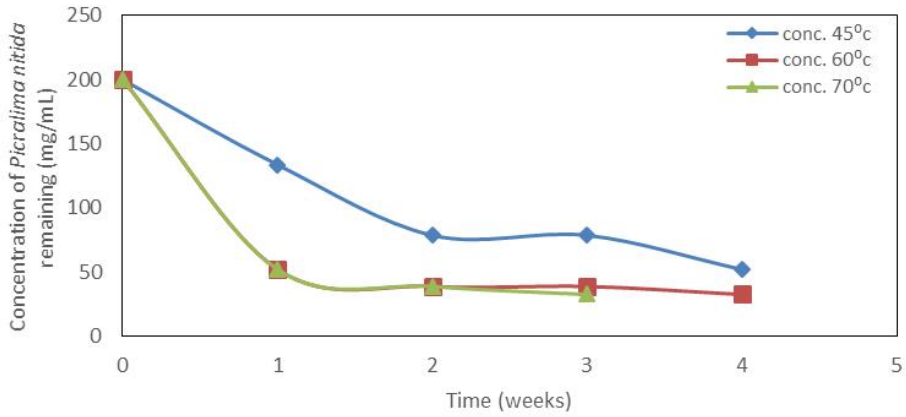

Fig. 9. Effect of storage time and temperature on *Pricralima nitida* concentration with *Pseudomonas aureginosa*

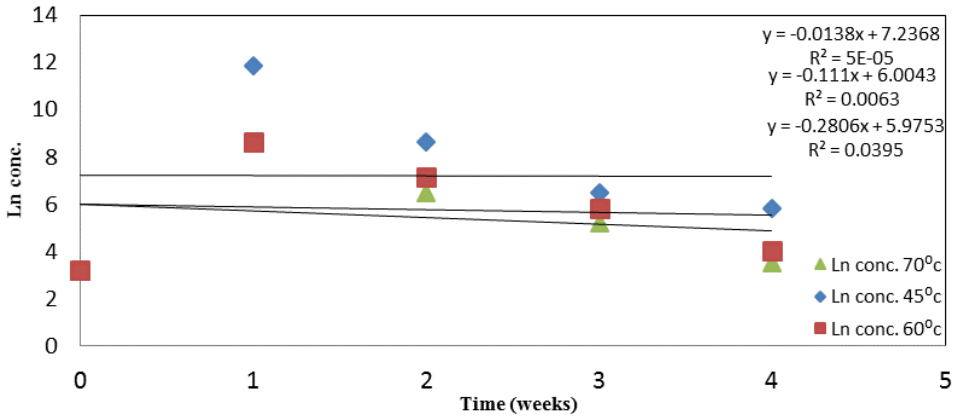

Fig. 10. Degradation kinetics of ciprofloxacin with *Bacillus subtilis*

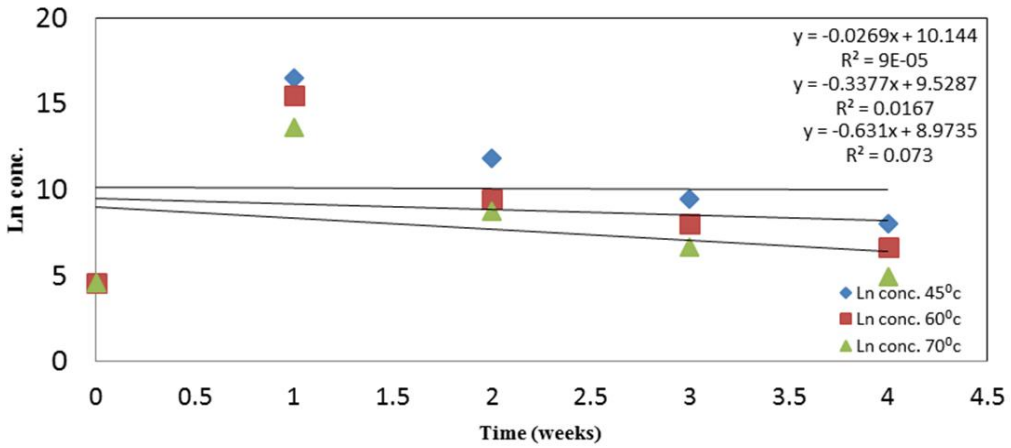

Fig. 11. Degradation kinetics of pefloxacin with *Bacillus subtilis*

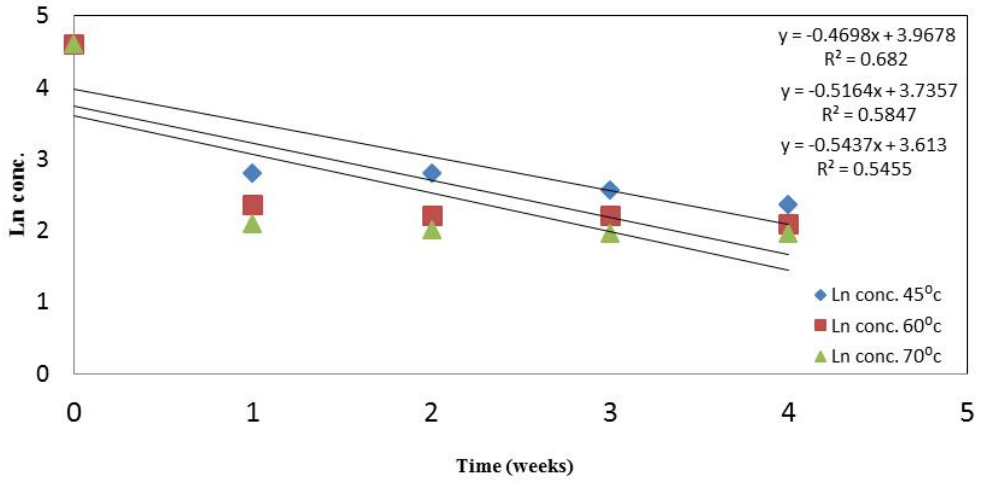

Fig. 12. Degradation kinetics of pefloxacin with *Samonella typhi*

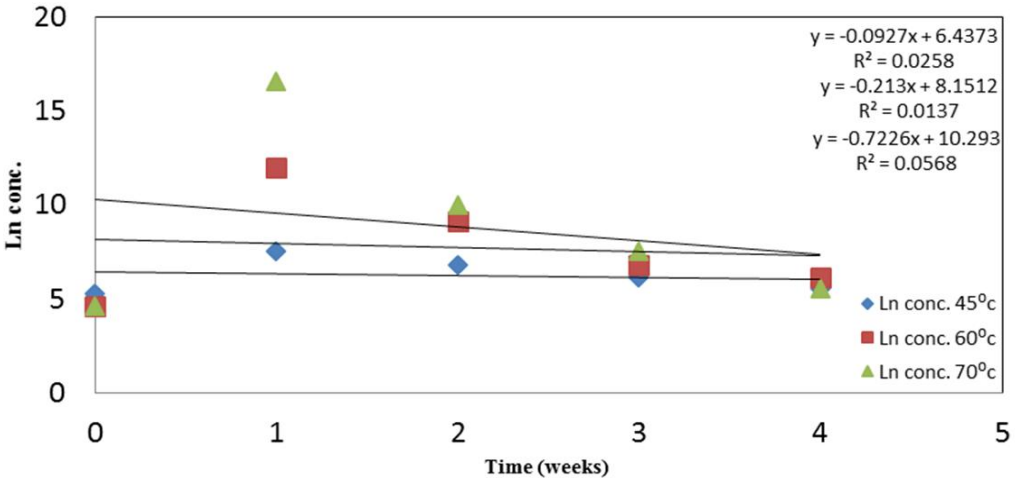

Fig. 13. Degradation kinetics of *Picralima nitida* with *Bacillus subtilis*

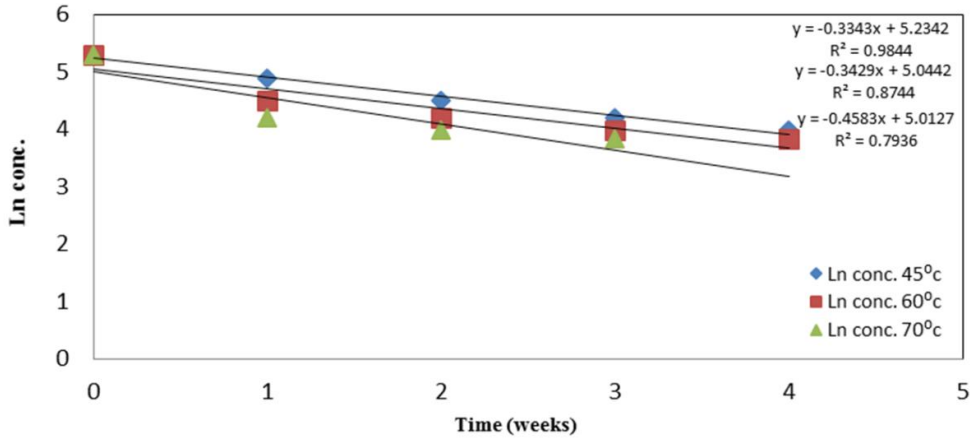

Fig. 14. Degradation kinetics of *Picralima nitida* with *Samonella typhi*

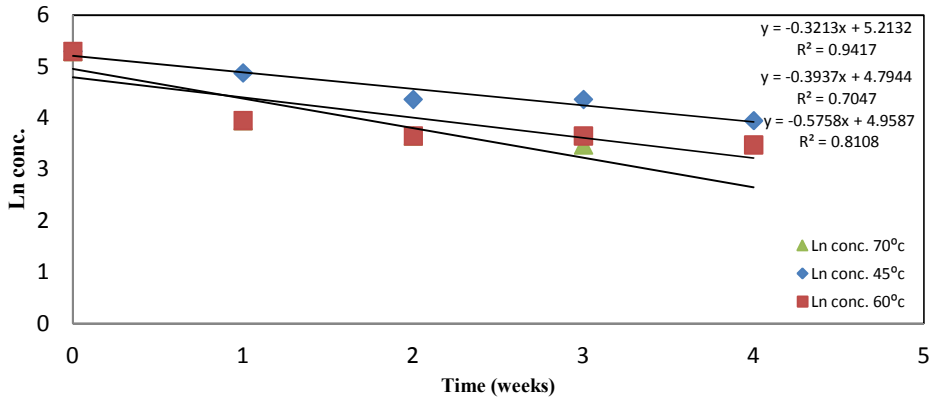

Fig. 15. Degradation kinetics of *Picralima nitida* with *Pseudomonas aureginosa*

3.5 Shelf-Life Determination

Arrhenius equation was used to determine the effect of temperature on the degradation kinetics. The relationship between temperature and rate of reaction was established using the Arrhenius equation by plotting Lnk values against 1/T (Figs.16 - 21). The value of *k* at 27°C (k_{27}) was extrapolated from the Arrhenius plot using Eq.2 [43]:

$$Ln\,K = Ln\,A - \frac{E_a}{RT} \quad (2)$$

Where K is the degradation rate constant, 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 (8.314J/mol.K).

The rate of degradation also depends on the activation energy of the chemical reaction [12]. The activation energy was evaluated from the slope of the plots (Figs. 16 - 21) using Eq. 3:

$$Slope = \frac{E_a}{R} \quad (3)$$

The calculated activation energies E_a for the degradation of the drug samples were found to be high (E_a> 50 kJ/mol.K), as shown in Table 1. The higher the E_a is, the less the degradation reaction is influenced by temperature; the result indicated that temperature contributed to the degradation of the drugs [44] but sensitivity activities to the bacteria also contributed to the degradation.

By substituting the values of k_{27} in the equation below the shelf-life of the drug substances were estimated:

$$t_{10\%} = \frac{Ln\,1.111}{k_{27}} = \frac{0.105}{k_{27}} \quad (4)$$

Where $t_{10\%}$ is the shelf life (the time required for 10% degradation of the drug) and k_{27} is the

degradation rate constant at 27°C. The shelf life of the drug substances is stated in Table 1. The shelf life of ciprofloxacin was found to be 80.31 wks against *Bacillus subtilis*; pefloxacin found to be 43.5 wks against *Bacillus subtilis* and 38.75 wks against *Samonella typhi*; *Picralima nitida* found tobe 5.83 wks against *Bacillus subtilis*, 0.41 weeks against *Bacillus subtilis* and 0.52 wks against *Pseudomonas aureginosa*.

Half-life, $t_{1/2}$of the drug substances (the period of time required for the concentration of drug to be reduced by one-half of a given concentration) were also estimated at 27°C as follows:

$$t_{1/2} = \frac{\text{Ln } 2}{k_{27}} = \frac{0.693}{k_{27}} \quad (5)$$

The half-life of the drug substances is stated in Table 1. The half-life of ciprofloxacin was found to be 533.08 wks against *Bacillus subtilis*; pefloxacin found to be 288.75 wks against *Bacillus subtilis* and 1.65 wks against *Samonella typhi*; *Picralima nitida* found to be 38.72 wks against *Bacillus subtilis*, 2.69 wks against *Bacillus subtilis* and 3.45 wks against *Pseudomonas aureginosa*.

3.6 Q_{10} Method for Shelf-Life Estimation

Q_{10}, an approach by Simonelli and Dresback [45] is the factor by which rate constant increases for a 10°C temperature increase. It is the ratio of two different reaction rate constants. Commonly used Q_{10} values of 2, 3 and 4 relate to the energy of activation of reaction.

$$Q_{10} = \frac{K_{(T+10)}}{K_T} \quad (6)$$

$$Q_{10} = exp\left[\frac{-E_a}{R}\left(\frac{1}{(T+10)} - \frac{1}{T}\right)\right] \quad (7)$$

When

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 the most likely estimate.

$Q_{10}$is used in the prediction and estimation of shelf life/ expiration date of drug substances at varying storage temperatures (Table 2) and is independent of the reaction order.

$$t_{90}(T_2) = \frac{t_{90}(T_1)}{Q_{10}^{(T_2-T_1)/10}} \quad (8)$$

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

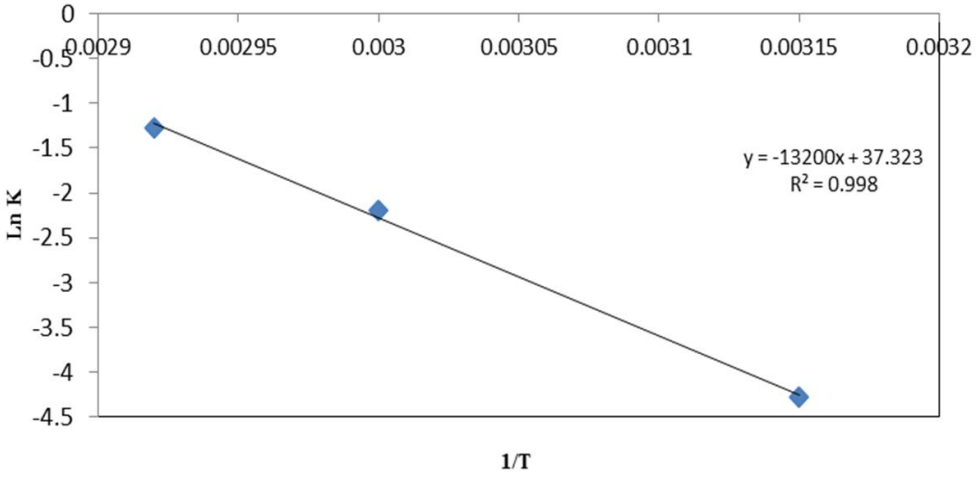

Fig. 16. Arrhenius plot for ciprofloxacin with *Bacillus subtilis*

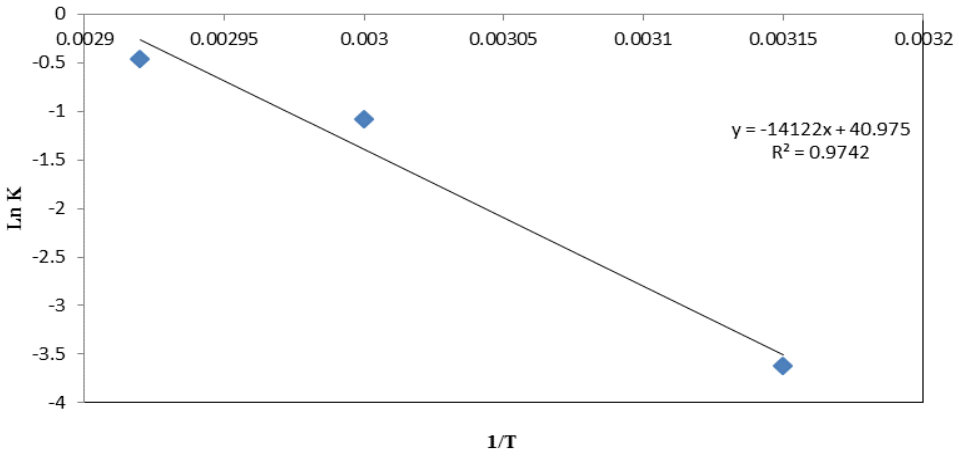

Fig. 17. Arrhenius plot for pefloxacin with *Bacillus subtilis*

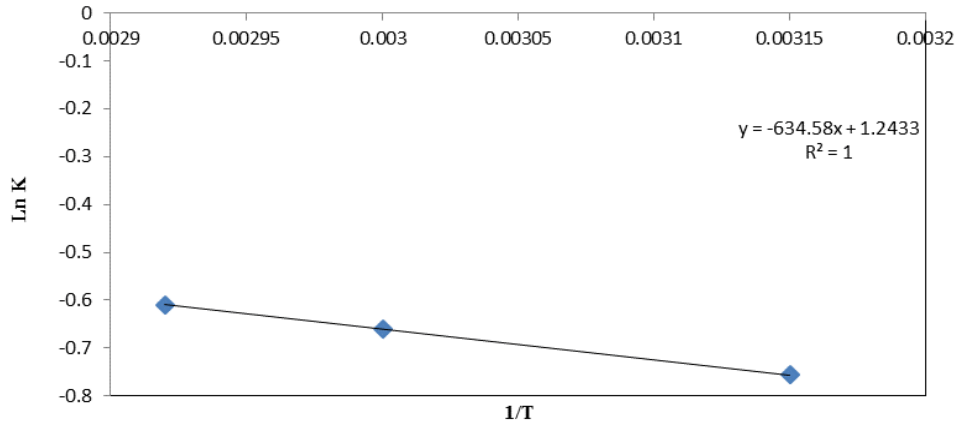

Fig. 18. Arrhenius plot for pefloxacin with *Samonella typhi*

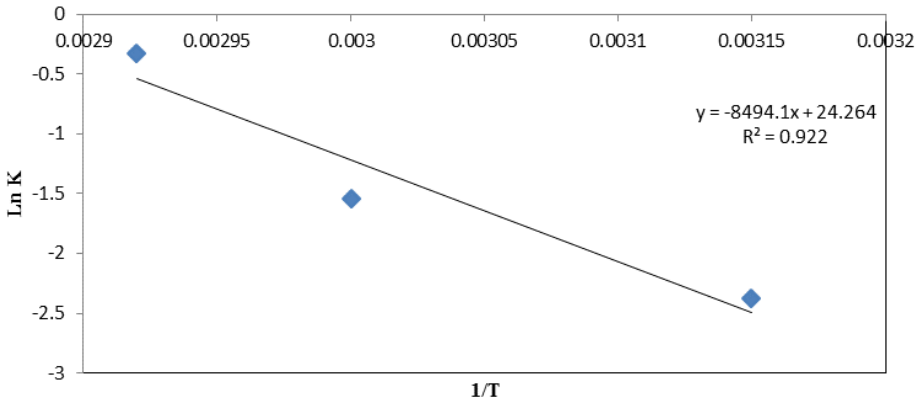

Fig. 19. Arrhenius plot for *Picralima nitida* with *Bacillus subtilis*

Table 1. Degradation rate constants at different temperatures, shelf-life, and half-life of ciprofloxacin, pefloxacin and *Picralima nitida*

Drug substance	Bacteria	Temp.(°C)	K_1(week-1)	Absolute Temp. (K)	1/T	LnK	Arrhenius factor(A)	Activation energy, E_a (kJ/mol.K)	Shelf-life at 27°C (wks)	Half-life at 27°C (wks)	Q_{10}
ciprofloxacin	*Bacillus subtilis*	27	0.0013	300	0.00333	-6.633					
		45	0.0138	318	0.00315	-4.28309	1.62×10^{16}	109.75	80.31	533.08	3.96
		60	0.111	333	0.003	-2.19823					
		70	0.2806	343	0.00292	-1.27083					
pefloxacin	*Bacillus subtilis*	27	0.0024	300	0.00333	-6.05125					
		45	0.0269	318	0.00315	-3.61563	6.24×10^{17}	117.41	43.5	288.75	4.36
		60	0.3376	333	0.003	-1.08589					
		70	0.631	343	0.00292	-0.46045					
	Salmonella	27	0.419	300	0.00333	-0.86985					
	typhi	45	0.4698	318	0.00315	-0.75545	3.47	5.28	0.25	1.65	1.07
		60	0.5164	333	0.003	-0.66087					
		70	0.5437	343	0.00292	-0.60936					
Picralima nitida	*Bacillus subtilis*	27	0.0179	300	0.00333	-4.02135					
		45	0.0927	318	0.00315	-2.37839	3.45×10^{10}	70.62	5.83	38.72	2.42
		60	0.213	333	0.003	-1.54646					
		70	0.7226	343	0.00292	-0.3249					
	Salmonella	27	0.2579	300	0.00333	-1.3553					
	typhi	45	0.3343	318	0.00315	-1.09572	1.49×10^{1}	10.12	0.41	2.69	1.14
		60	0.3429	333	0.003	-1.07032					
		70	0.4583	343	0.00292	-0.78023					
	Pseudomonas	27	0.201	300	0.00333	-1.60458					
	aureginosa	45	0.3213	318	0.00315	-1.13538	5.65×10^{2}	19.83	0.52	3.45	1.28
		60	0.3937	333	0.003	-0.93217					
		70	0.5758	343	0.00292	-0.55199					

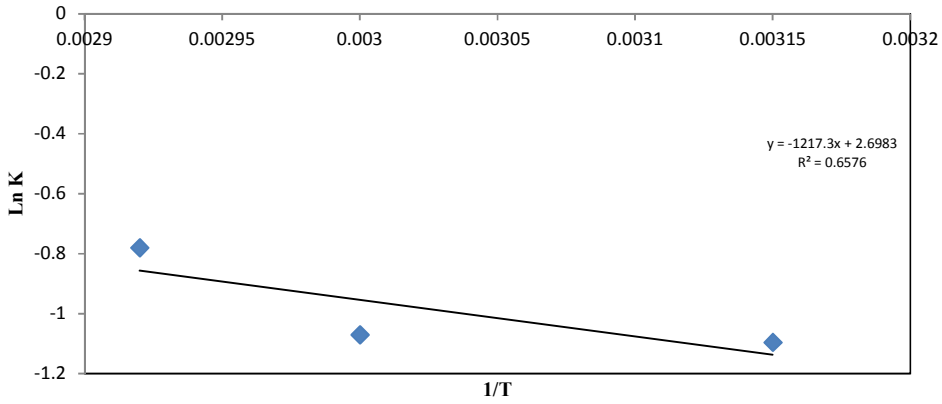

Fig. 20. Arrhenius plot for *Picralima nitida* with *Samonella typhi*

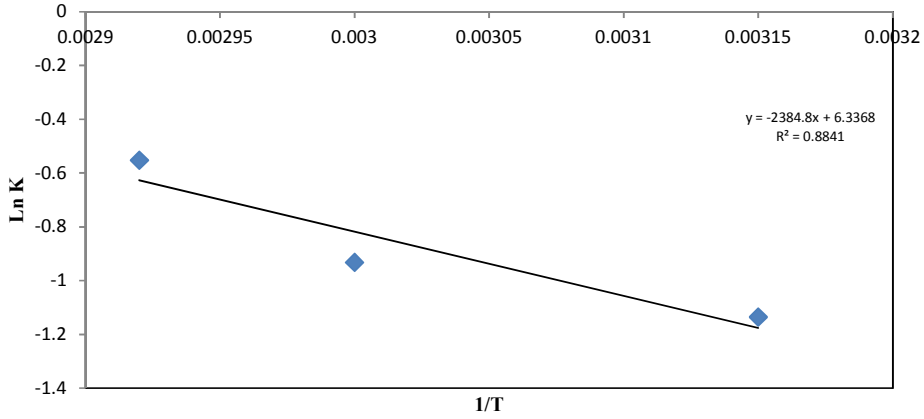

Fig. 21. Arrhenius plot for *Picralima nitida* with *Pseudomonas aureginosa*

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

Drug substance	Bacteria	Q_{10}	Shelflife at 27°C (wks)	Shelflife at 4°C (wks)	Shelflife at 37°C (wks)
Ciprofloxacin	*Bacillus subtilis*	3.96	80.31	1903.13	20.28
Pefloxacin	*Bacillus subtilis*	4.36	43.5	1286.2	9.97
	Salmonella typhi	1.07	0.25	0.29	0.23
Picralima nitida	*Bacillus subtilis*	2.42	5.83	44.51	2.41
	Salmonella typhi	1.14	0.41	0.55	0.35
	Pseudomonas aureginosa	1.28	0.52	0.91	0.41

4. CONCLUSION

The research was based on the comparative study on the shelf life ($t_{10\%}$) of *Picralima nitida* (herbal drug) and two orthodox drugs (ciprofloxacin and pefloxacin). Bio-based concentration-activity relationship technique was used for the stability/shelf life study. Storage time (1, 2, 3 and 4 wks) and temperature (45, 60 and 70°C) effects on the concentration of the drugs were also investigated via the accelerated stability studies. First-order degradation kinetics constant, k_1 and half-life, $t_{1/2}$ were also evaluated. All the drugs proved to be broad-spectrum antibiotics and their concentrations were found to decrease with increase in storage time and temperature. The activity of the orthodox drugs (fluoroquinolones) against *Escherichia coli* and

Staphylococcus aureus were immeasurable even at low dose. Ciprofloxacin proved to be more active and stable but loss its activities against the organisms at stressed condition respectively; but *Picralima nitida* retained its activity more at stressed condition owing to the presence of active metabolites. Using bio-based concentration-activity relationship technique, the shelf life of ciprofloxacin, pefloxacin and *Picralima nitida* can be evaluated based on their inhibitory activity but varies due to sensitivity activities on different bacteria.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly used products in this area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because they do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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