Journal of Advanced Engineering Research
ISSN: 2393-8447
Volume 7, Issue 1, 2020, pp.11-19

Reliability Prediction Model Patterns for Neem Leaf Phytochemicals at Varying Temperature

Orlando Ketebu*, Yousuo Digieneni, Owologba Gladys Ome

Department of Chemical Engineering, Niger Delta University, Amassoma, Bayelsa State, Nigeria

*Corresponding author email: orlandok2@yahoo.com

ABSTRACT

Neem leaf extract phytochemicals are known for their biochemical and medicinal purposes in treating sickness and diseases. The model patterns with which the phytochemicals (alkaloids, flavonoids, saponins, tannins, and terpernoids) changes with temperature is of importance because there are no model relationships to show how this phytochemicals changes with temperature and their efficacy and activities depends on temperature. This paper predicts the relationship pattern of neem leaf extract phytochemicals at temperatures 25°C, 40°C, 60°C, 80°C and 100°C respectively. The experimental results showed that 25°C and 40°C had alkaloids, flavonoids, saponins and terpenoids at medium concentrations and tannins at high concentration and above 60°C they diminish. Alkaloids, tannins, saponins and terpenoids followed a second order polynomial model pattern with reliability prediction (R^2) of 0.9964, 0.9971, 0.9949, and 0.9939 while flavonoids varied linearly with R^2 of 0.9955 respectively. The pH and specific gravity of the neem leaf extract decreases with increasing temperature while conductivity increases, all in a second order polynomial form with R^2 values of 0.9929, 0.9988 and 0.9969 respectively. The high values of reliability prediction showed that the model equations perfectly predict the changes taking place and can be used as a model for future scale up processes.

Keywords - Neem extract, model relation, phytochemicals, Reliability prediction, Temperature

1. INTRODUCTION

The use of herbal medicine from medicinal plants to treat sicknesses and diseases has been on since time immemorial before the introduction of modern medicine. The value of medicinal plants has been accepted as a potential source of bioactive compounds due to their phytochemical components [1]. Recent researches have shown that many phytochemicals could protect humans from diseases and sickness such as malaria, diabetes, cancer, hypertension, diarrhea, tumor, viral infection etc., [1-5]. But there are no research works on the prediction patterns which some of these phytochemicals follow when heated and their subsequent change with temperature. Since phytochemicals in plants are responsible for biochemical and medical activities in human when applied therapeutically.

Phytochemicals are biologically active chemical compound in plants such as alkaloids (Alk), flavonoids (flav), tannins (Tan), saponins (Sap), terpenoids (Terp), phenols (Phe) etc. Phytochemicals occur naturally in plants and are not regarded as plants essential nutrients, but are responsible for protecting plant cells against hazards that may occur in the form of stress, ultraviolet exposure, pollution and pathogenic attacks and are also known for their health benefits in humans when consumed [2].

Alkaloids are nitrogen base organic compounds found in plant and aid the growth, reproductive and metabolic processes in plants. They have anti-arrhythmic and anti-hypertensive effects in human and also possess anticancer and antimalarial activities [4, 6, 7, 8, 9].

Tannins are phenolic compounds found in plants. They are used as astringents against diarrhea, as diuretics and as anti-inflammatory, anti-diabetic, antiseptic and antioxidants [2, 4, 10, 11, 12].

Terpenoids (isoprenoids) are lipids derived from five carbon isoprene units found in plants. Terpenoids are known for their medicinal properties such as antitumor and anti- inflammatory activity, anticarcinogenic, antimalarial, antiulcer, antimicrobial, hepatidal, diuretic and anticancer properties [13-16].

Flavonoids are phytonutrient compounds found in plants and are responsible for the brilliant colours in fruits and vegetables. Flavonoids are known to improve cardiac function, decrease cholesterol level and also regulate hypertension [4, 17, 18]. They are also known to acts as antioxidants and protect human body from free and non-free radicals [17-18].

Saponins are water loving chemical compounds found in plants and acts as part of plants' defense system. In human they act as antimicrobial agent fighting against fungi, bacteria and viruses. They are known for their antitumor properties, immunostimulant, hypoglycemic, antifungal, antibacterial, antiprotozoal and anticarcinogenic properties [19-20].

Plants leaf such as neem leaf also known as dongoyaro in Nigeria and other plants such as bitter leaf, pawpaw leaf, plantain leaf etc. have phytochemical properties useful for human health. Neem tree is one of the medicinal plants with numerous health benefits known to man. It is a fast growing tree with drought resistant properties found in India, Africa and America. Its leaves are purple-red when young and turns medium green colour when mature.

In the therapeutic use of this plant traditionally, the phytochemicals of this plant is obtained by squeezing the leaves in water at room temperature or heating or boiling the leaves in water. This paper predicts the model patterns the phytochemicals concentration follows during changing temperature.

Other research work carried out on Neem leaf extract includes its use as an inhibitor for corrosion of steel in acidic environment and in removing oil from waste water [21-22].

2. METHODOLOGY

2.1 Materials and Equipment

Wheatman filter paper (varying no.), UV/VIS spectrophotometer (Jenway 6500), Weighing balance (Scientech digital (0.01-210 g)), Conical flasks (250 ml), Soxhlet extractor and thimbles, Rotary evaporator (Buchii Product), Desiccator, Beakers (250 ml and 500 ml.), Buchner funnel and suction pump, Muffle furnace (1000°C), Pressure boiler (700°C), 40% and 2 M Sodium Hydroxide (NaOH) solution, Petroleum ether (Variable boiling range.), 0.1 M and 2 M Concentrated Sulfuric acid (H_2SO_4), 0.1 M Boric acid, mixed indicator of methyl red and methylene blue, 0.1 M Hydrochloric acid (HCl), Sodium sulphate (Na_2SO_4 crystals), Copper sulphate ($CuSO_4$ crystals), Mayer's reagent, Dragendorff's reagent, Ethyl acetate, Aluminium chloride, Ammonia solution, Olive oil, Ethanol (45%), Ferrous sulphate, Ferrous chloride, Lead acetate, Bromine water, Fehling solution A and B, Chloroform, cotton wool, aluminum foil. Tannic acid solution, saturated sodium carbonate solution, methanol, n-butanol, Sodium chloride (NaCl), Diethyl ether.

2.2 Method

2.2.1 Preparation of plant extract

Freshly plucked neem stems were brought to the laboratory and washed under running water and distilled water to remove external impurities. The leaves were plucked off from the branches and blended in a laboratory blender. 500 grams of blended leaves were then soaked in one liter of distilled water. The mixture of blended leaves and water was strained through a muslin cloth. The viscous extract obtained was used for the phytochemical analysis at the different temperatures.

2.2.2 Qualitative procedures for the analysis of phytochemicals

2.2.2.1 Alkaloids identification test

0.2 grams of neem extract was measured and mixed with 2% hydrochloric acid (5ml). The mixture was placed in a steam bath and boiled for five minutes. The boiled mixture was cooled to room temperature before filtration. 1 ml of the filtrate was measured and placed in three different test tubes labeled A, B and C respectively. Mayer's reagent (two drops) was added to test tube A which results in the formation of a cream like white precipitate indicating the presence of alkaloids. Further confirmation of alkaloids presence in the filtrate was carried out by adding Dragendorff's reagent (two drops) in test tube B containing 1ml of the filtrate. The mixture forms red precipitate which confirms alkaloids presence in the filtrate.

2.2.2.2 Flavonoids identification test

Qualitative analysis for flavonoid in neem leaf extract was carried out by measuring 0.5 gram of neem leaf extract and placing it inside a test tube followed by the addition of ethyl acetate solution (10 ml). The mixture was filtered and 4 ml of the filtered extract was mixed with 1% aluminium chloride solution (1ml) in a test tube and the reaction proceeded for ten minutes. The

appearance of yellowish colour showed that flavonoid was present in the filtrate.

2.2.2.3 Saponins identification test

0.1 gram of neem leaf extract was measured and mixed with 5 ml of distilled water in a beaker and boiled for five minutes. The hot mixture was filtered followed by the addition of olive oil (two drops) to 1 ml of the filtrate placed in a test tube. The mixture was agitated and an emulsion was formed. Saponins presence was further confirmed by agitating another 1 ml of the filtrate mixed with 4 ml of water in a test tube. A stable frothing was formed on standing.

2.2.2.4 Tannins identification test

0.2 grams of powdered neem leaf extract was mixed with 45% ethanol (5 ml) and boiled in a water bath for five minutes. The mixture filtered after allowing it to cool to room temperature. 1 ml of the filtrate was measured and placed in a test tube and lead acetate solution added (3 drops). The appearance of gelatinous precipitate shows the presence of tannins in the filtrate. This was further confirmed by measuring 1 ml of the filtrate and mixing it with bromine water (0.5 ml). A light brown precipitate was formed.

2.2.2.5 Terpenoids identification test

0.5 grams of neem leaf extract was measured and placed in a test tube followed by careful addition of chloroform (2 ml) and concentrated sulphuric acid (3 ml) respectively. A reddish-brown interface was formed which confirms terpenoids presence in the extract.

2.2.3 Quantitative procedures for the analysis of phytochemicals

2.2.3.1 Determination of Alkaloids

The procedure for quantitative analysis of alkaloid follows similar procedure adopted by Harbone gravimetric method [23] with modification. The procedure is as follows, 5 grams of powdered neem leaf extract was measured and placed inside a beaker (250 ml) followed by the addition of 200 ml acetic acid (10%). The mixture was covered with aluminum foil and allowed to stand for four hours before it was filtered. The extract was further concentrated to about ¼ of its initial volume in a water bath followed by drop wise addition of concentrated ammonium hydroxide to form precipitation. The precipitate was filtered using Wheatman filter paper and washed using dilute ammonium hydroxide solution. The residue was dried in an oven at 65°C until completely dried. It was then weighed as the alkaloids obtained. The percentage of alkaloid obtained was calculated using Equation 1, where A is the weight of measured neem leaf extract and B represents the weight of the dried precipitate.

$$\% \text{ Alkaloids} = \frac{A}{B} \times 100 \qquad 1$$

This procedure was repeated but this time the filtrate was subjected to heating at temperatures 40°C, 60°C, 80°C and 100°C respectively before calculating alkaloids percentage.

2.2.3.2 Determination of Flavonoids

Flavonoids analysis was carried out following similar method adopted by Bohm and Koupal-Abyazani [24] with modifications. In this method, 10 grams of finely grounded neem leaf extract was measured and placed in a beaker at room temperature and 100 ml aqueous methanol (80%) was added for extraction. After extraction, the extracts were filtered using Wheatman's filter paper. The filtrate was placed in a porcelain crucible and heated to dryness in a water bath. The dried filtrate was then weighed. Percentage flavonoids were obtained by applying Equation 2.

$$\% \text{ Flavonoids} = \frac{B}{S} \times 100 \qquad 2$$

The letter B is the weight of dried filtrate and S is the weight of grounded neem leaf extract. The process was repeated by heating the filtrate at 40°C, 60°C, 80°C and 100°C respectively before heating to dryness.

2.2.3.3 Determination of Saponins

Quantitative values for saponins were obtained following similar method adopted by Obadoni and Ochuko [25]. In the procedure finely grounded neem leaf extract was measured (10 grams) and placed in a conical flask (100 ml) followed by the addition of 20% ethanol solution (50 ml). The extract mixture was stirred continuously in a water bath regulated at 55°C for hours (approximately 4 hours) before filtration. Further extraction of the residue left after filtration was carried out by mixing the residue with an aliquot of ethanol solution. The extracts obtained from the two processes were mixed together to obtain 40 ml concentrate in a water bath regulated at 90°C. The resultant mixture was agitated with diethyl ether solution (10 ml) in a 250 ml separating funnel. Two layers were formed in the funnel. The aqueous layer was recovered from the funnel while the ether layer was

removed. Further extraction of the aqueous layer was carried out using 30 ml n-butanol mixed with aqueous sodium chloride twice. The extract solution was then heated to dryness using a water bath and further dried to constant weight in an oven. The percentage of saponins formed was calculated by applying Equation 3, where Wr is the weight of residue and Ws is the weight of the finely ground neem leaf extract..

$$\% \text{ Saponins} = \frac{W_r}{W_s} \times 100 \qquad 3$$

The procedure was repeated by combining both extract and heating them at temperatures 40°C, 60°C, 80°C, 100°C respectively before heating to dryness and calculating percentage saponins.

2.2.3.4 Determination of Tannins

The quantitative values for tannins were obtained by following similar method adopted by Pearson [26]. In the procedure, 1 gram of neem leaf extract was mixed with 10 ml of distilled water and agitated for ten minutes. The mixture was left to settle down for about thirty minutes and then centrifuged for another ten minutes at 3000 revolution per minute. An aliquot of the supernatant (2.5 ml) was measured and placed in 50 ml volumetric flask. Another aliquot of 2.5 ml aqueous tannic acid was measured and placed in another volumetric flask. Folin-Denis reagent (1 ml) was added to the volumetric flasks containing the supernatant and tannic acid solution respectively. Saturated sodium trioxocarbonate solution (2.5 ml) was added to the flasks and the flasks placed in an incubator for ninety minute. Spectrophotometer (UV/VIS) analysis was carried out to determine the absorbance at 250 nm. The percentage tannin content was obtained from Equation 4.

$$\% \text{ Tannins} = \frac{A_n}{A_s} \times C \times \frac{100}{W} \times \frac{V_f}{V_a} \qquad 4$$

Where; W = weight of neem leaf extract, A_n = absorbance of supernatant, A_s = absorbance of tannic solution, C = concentration of standard tannin solution, V_f = total volume of extract used, and V_a = volume of analyzed extract. The procedure was repeated with the supernatant extract heated to temperatures 40°C, 60°C, 80°C and100°C respectively.

2.2.3.5 Determination of Terpenoids

In the determination of terpenoids, the Ferguson's method was adopted [27]. In this method 1.0 grams of finely grounded neem leaf extract was placed in a test tube and methanol added. The mixture was left for 24 hours before filtering using cotton wool. After filtering, the cotton wool was washed with fresh methanol (10 ml). The filtrate was removed and placed in a volumetric flask where further extraction was carried out using petroleum ether (50 ml). The extracts were evaporated to dryness with constant weight using water bath. Percentage terpenoids were obtained by applying equation 5, where Wr is the weight of residue (dried extracts) and Ws is the weight of finely grounded neem leaf extracts.

$$\% \text{ Terpenoids} = \frac{W_r}{W_s} \times 100 \qquad 5$$

The procedure was repeated with the extraction of the filtrate at temperatures 40°C, 60°C, 80°C and 100°C respectively before evaporating to dryness.

3. RESULTS AND DISCUSSION

Table 1 showed the qualitative analysis of neem leaf extract for alkaloids, flavonoids, saponins, tannins and terpenoids at increasing temperatures in triplicates. In the table, all the phytochemicals were present at room temperature 25°C and at 40°C. These two temperatures showed that tannins had high concentrations while alkaloids, flavonoids and terpenoids are of medium concentrations and saponins had low concentration. The table also showed that as the temperature increased to 60°C, the concentration of tannins reduces to medium while that of alkaloids, saponins, terpenoids and flavonoids reduces to low concentrations. At temperatures above 60°C, the phytochemical concentration of the neem leaf extract reduced to low concentration and some samples showed negative indicating absence of phytochemicals.

This result is corroborated with the quantitative analysis of the neem leaf phytochemicals shown in Table 2. In the table, Tannins had the highest percentage values at room temperatures (25°C) followed at 40°C. The other phytochemicals (alkaloids, flavonoids, terpenoids, saponins, tannins) had medium values at these two temperatures and also at 60°C. The value of these phytochemicals diminishes as the temperature increases. A sharp drop in the concentration of the phytochemicals was observed at 80°C and 100°C with the minimum values at 100°C.

The results from the two Tables (1 and 2) showed that the best temperature for neem leaf extract phytochemicals is at room temperature or at 40°C. This

is because at these temperatures all the phytochemicals screened were present in high and medium amount that can function as biochemical or medicinal agents.

Situations where heating is involved to extract the phytochemicals and to minimize bacteria's contamination, neem leaf extract should not be heated above 60°C. Because at this temperature the phytochemicals are still present in considerable amount and can function effectively relative to room temperature and 40°C. Heating at temperatures above 60°C reduces the amount of phytochemicals in the leaf extract which in turn reduces the efficacy of neem leaf.

Table 1 Qualitative phytochemical screening of Neem leaf extract at varying temperature (+++ High concentration, ++ Medium concentration, + Low concentration)

Temp. (°C)	Triplicate runs	Alkaloids	Flavonoids	Saponins.	Tannins.	Terpenoids.
25	1	++	++	+	+++	++
	2	++	++	+	+++	++
	3	++	++	+	+++	++
40	1	++	++	+	+++	++
	2	+	+	+	++	++
	3	++	++	+	+++	++
60	1	+	+	+	++	++
	2	+	+	+	++	++
	3	+	+	+	+	+
80	1	+	+	+	+	+
	2	+	+	-	+	-
	3	-	+	+	-	+
100	1	-	+	-	+	-
	2	+	-	+	-	-
	3	-	+	-	+	+

Table 2 Quantitative phytochemical screening of Neem leaf extracts showing percentage composition (%) at varying temperature

Temp. (°C)	Triplicate runs	Alkaloids	Flavonoids	Saponins.	Tannins.	Terpenoids.
25	1	0.65	0.78	1.68	7.82	1.23
	2	0.66	0.78	1.70	7.80	1.20
	3	0.64	0.78	1.67	7.78	1.25
	Average	0.65	0.78	1.68	7.80	1.23
40	1	0.60	0.73	1.57	7.60	1.21
	2	0.62	0.71	1.54	7.61	1.20
	3	0.61	0.72	1.53	7.59	1.22
	Average	0.61	0.72	1.55	7.60	1.21
60	1	0.56	0.63	1.34	6.66	1.18
	2	0.58	0.64	1.36	6.65	1.16
	3	0.57	0.62	1.35	6.66	1.17
	Average	0.57	0.63	1.35	6.66	1.17
80	1	0.50	0.56	1.04	5.52	1.01
	2	0.50	0.57	1.02	5.56	1.00
	3	0.46	0.58	1.01	5.54	1.00
	Average	0.49	0.57	1.02	5.54	1.00
100	1	0.38	0.48	0.80	4.25	0.82
	2	0.39	0.46	0.76	4.23	0.81
	3	0.37	0.47	0.78	4.24	0.82
	Average	0.38	0.47	0.78	4.24	0.82

Table 3 shows the pH, specific gravity (Sp.Gr) and conductivity (Cond) of neem leaf extract phytochemicals at increasing temperatures. It is observed from the table that the pH and specific gravity are decreasing as the temperature increases while the conductivity is increasing with increasing temperatures. The decreasing pH showed that the neem phytochemicals are becoming more acidic in solution as the temperature increases and the decreasing specific gravity indicates that the phytochemicals in solutions becomes less dense than water as the temperature increases. On the other hand, the conductivity of the neem leaf phytochemicals increases with increasing temperature due to the presence of ions resulting from the acidic nature of the extract solution.

Table 3 pH, Conductivity and specific gravity of neem leaf juice at varying temperatures.

Temp. (°C)	Triplicate runs	pH	Conductivity (µS)	Specific Gravity
25	1	6.25	3320	1.068
	2	6.24	3318	1.070
	3	6.26	3324	1.066
	Average	6.25	3321	1.068
40	1	6.08	3370	1.041
	2	6.10	3368	1.040
	3	6.06	3371	1.040
	Average	6.08	3370	1.040
60	1	5.80	3840	1.018
	2	5.82	3845	1.016
	3	5.84	3842	1.017
	Average	5.82	3842	1.017
80	1	5.51	4486	1.000
	2	5.50	4500	0.999
	3	5.50	4486	1.008
	Average	5.50	4491	1.002
100	1	4.85	5820	0.998
	2	4.80	5818	0.994
	3	4.83	5800	0.996
	Average	4.83	5813	0.996

These relationships were graphically presented and the points on the graphs showed the model relationship on how the phytochemicals responds to varying temperatures. Fig. 1 shows the plot of average quantitative values of alkaloids percentage against varying temperatures. The alkaloid concentrations were found to decrease in a second order polynomial form with increasing temperature. This was predicted as shown in the model equation from the relationship in equation 6.

$$Alk = -3e^{-05}T^2 + 0.0004T + 0.6542 \qquad 6$$

Equation 6 has reliability prediction (R^2) of 0.9964 which can be easily approximated to 1 and showed that the model equation perfectly predicts the change in percentage alkaloids with varying temperature.

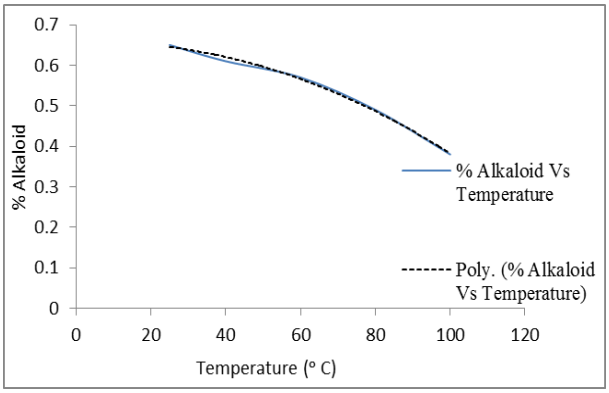

Fig. 1 Percentage Alkaloids against Temperature

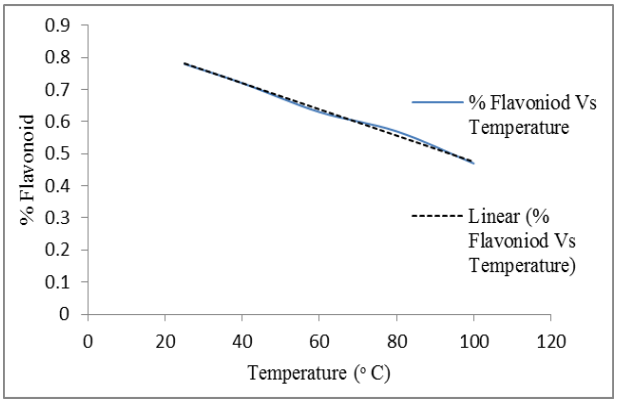

Fig. 2 Percentage flavonoids against Temperature

Fig. 2 shows the plot of percentage flavonoids against varying temperature and the model relationship predicts that percentage flavonoids varies linearly with increasing temperatures. The predicting equation is shown in equation 7 with $R^2$of 0.9955 approximately 1. This also indicates that the model relationship perfectly predicts the change in percentage flavonoids with varying temperature.

Similar model equations were obtained for plot of percentage saponins against temperature in Fig. 3, percentage tannins against temperature in Fig. 4 and percentage terpenoids against temperature in Fig. 5. The results showed that percentage saponins varied with second order polynomial form with increasing temperature and had $R^2$of 0.9949 as shown in equation 8. Tannins and terpenoids also varied with second order polynomial form with varying temperature as shown in the model equation 9 and 10 with R^2 of 0.9971 and 0.9939 respectively. These high values of reliability predictions for saponins, tannins and terpenoids approximately 1, showed that the model equations predict perfectly the relation of these phytochemicals with varying temperatures.

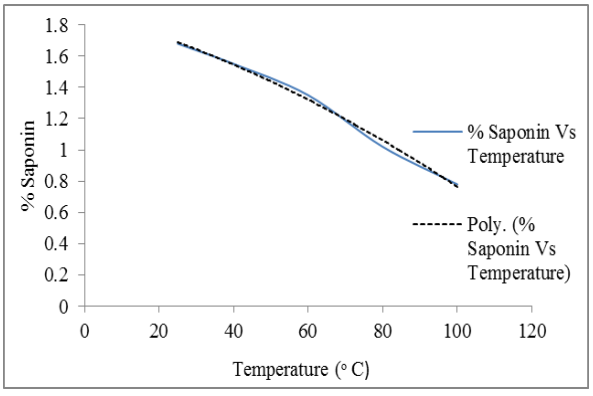

Fig. 3 Percentage Saponins against Temperature

$$Flav = -0.0041T + 0.8812 \quad (7)$$

$$Sap = -5e^{-05}T^2 - 0.0066T + 1.8828 \quad (8)$$

$$Tan = -0.0004T^2 - 0.0011T + 8.1323 \quad (9)$$

$$Terp = -8e^{-05}T^2 - 0.0048T + 1.1589 \quad (10)$$

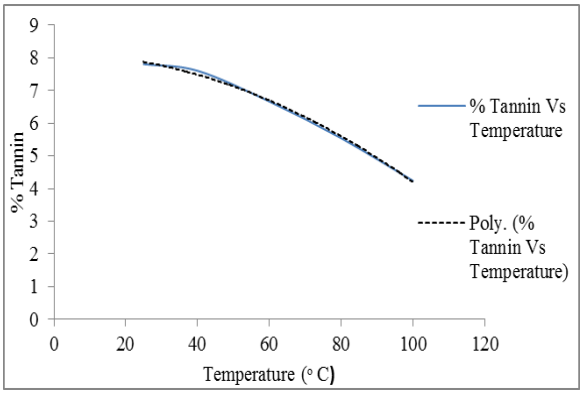

Fig. 4 Percentage Tannins against Temperature

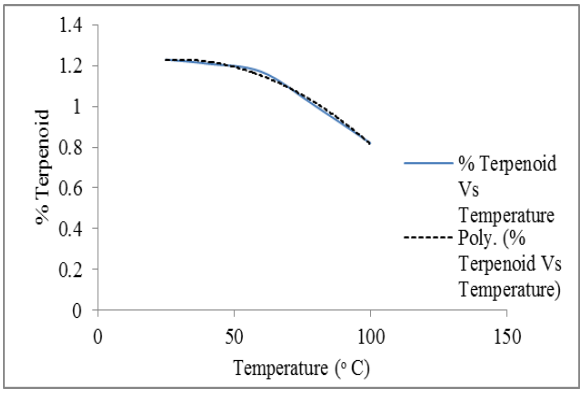

Fig. 5 Percentage Terpenoids against Temperature

Predicting model equation for change in pH, conductivity and specific gravity for the neem leaf phytochemicals was also carried out. Equation 11, 12 and 13 showed that the pH, specific gravity and conductivity of the neem leaf phytochemicals varied polynomially with changing temperature with R^2 of 0.9929, 0.9988 and 0.9969 respectively. Fig. 6, 7 and 8 showed the graphical relationships of pH, specific gravity and conductivity against varying temperature respectively. The prediction reliability values for pH, specific gravity and conductivity showed that the equation perfectively predicts the relationships.

$$pH = -0.0002T^2 + 0.0054T + 6.2042 \quad (11)$$

$$Sp.Gr = -e^{-05}T^2 - 0.0025T + 1.1223 \quad (12)$$

$$Cond. = 0.5017T^2 - 30.07T + 3771.6 \quad (13)$$

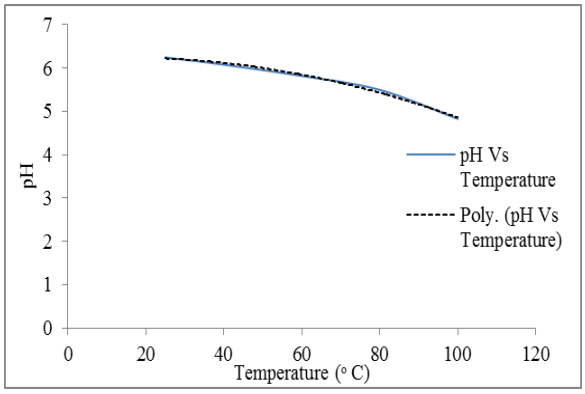

Fig. 6 pH against Temperature

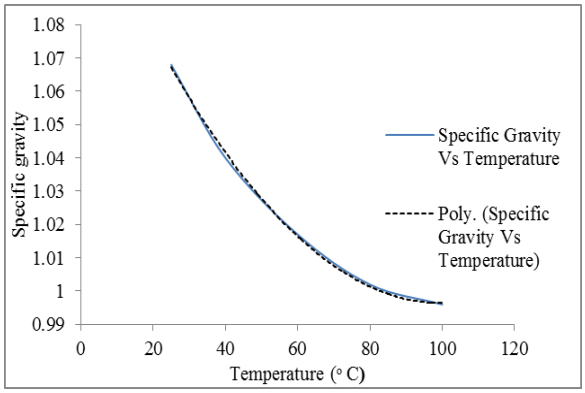

Fig. 7.Specific gravity against Temperature

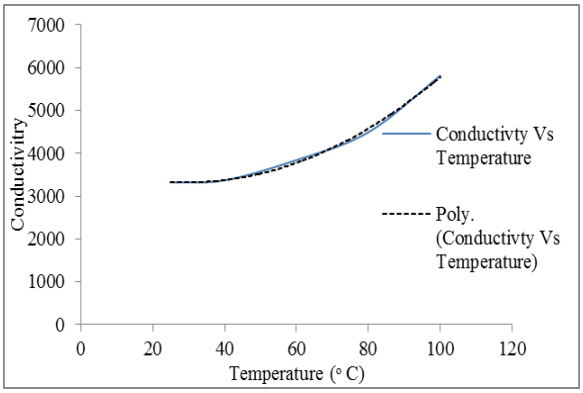

Fig. 8 Specific gravity against Temperature

4. CONCLUSION

The phytochemical analysis data for alkaloids, flavonoids, saponins, tannins and terpenoids for neem leaf extract reveals that the phytochemicals concentration decreases with increasing temperature. The neem leaf extract extracted at 25°C and 40°C had high and medium phytochemical concentrations. Moderate concentration of phytochemicals were observed at 60°C and above 60°C, the phytochemicals concentration diminishes. The phytochemicals alkaloid, tannin, saponin and terpenoid concentrations varied with temperature following a second order polynomial pattern with R^2 of 0.9964, 0.9971, 0.9949, 0.9939 respectively. Flavonoid varied linearly with changing temperature with R^2 of 0.9955. The reliability prediction values approximately 1 indicates that the model equations obtained predicts perfectively the relationship between the phytochemicals concentration and temperature change. The pH and specific gravity of the juice also decreases with increasing temperature following a second order polynomial pattern while the conductivity increases as temperature increases in a second order polynomial form too. Their reliability prediction values are 0.9929, 0.9988 and 0.9969 respectively. The decreasing pH and specific gravity and increasing conductivity showed that as temperature increases the phytochemicals in solution become more acidic and less dense than water and more conducting as an electrolyte.

REFERENCES

[1] K. Biswas, I. Chattopadhyay, R.K. Banerjee, U. Bandyopadhyay, Biological activities and medication properties of neem (Azadirachta indica), *Curriculum-Science*, 82, 2002, 1336-1345.

[2] S. Mamta, S. Jyoti, N. Rajeev, S. Dhamendra, G. Abhishek, Photochemistry of medicinal plants, *Journal of Pharmacognosy Photochemistry*, 1, 2013, 168-182.

[3] M. Shamilla, M. Rajeswari, P. Vijayashalini, Comparism of qualitative phytochemical analysis of amarantus polygonoides L. and amarantus Viridis L, *International Journal of Applied Research*, 3, 2017, 280-283.

[4] O. T. Ogunmefun, Phytochemicals- God's endowment of curative power in plants, phytochemicals – source of antioxidants and role in disease prevention, *Intechopen*, 2018, 77423.

[5] C.A. Hassan, A.H.Wafaa, A.A.T.Hanan, Invitro antitumor and antiviral activities of seeds and leaves of Neem (Azadirachta indica) extracts, *International Journal of Academic Research*, 2, 2010, 165-171.

[6] J. Kurek, Introductory chapter: Alkaloids- Their importance in nature nad for human life, *IntechOpen*, DOI: 105772/intechopen, 2019.

[7] V. Somsak, S.Chachiyo, U. Jaihan, and S. Nakinchat, Protective effect of aqueous crude extract of neem leaves on plasmodium berghei-induced renal damage in mice, *Journal of Trorpical Medicine*, 2015.

[8] B. Nouredine, Pharmacological activity of alkaloids: A review. 1. *Asian Journal of Botany*, 1, 2018.

[9] I.J, Udeinya, N. Brown, E.N. Shu, F.I. Udeinya and I. Quakeyie, Fraction of an antimalarial neem-leaf extract have activities superior to chloroquin and are gametocytocidal, *Annals of Tropical Medicine and Parasitology*, 100, 2006, 17-22.

[10] S. Pankaj, T. Lokeshwar, B. Mukesh, B. Vishnu, Review on Neem: Thousand problems one solution, *International Research Journal of Pharmacy*, 2, 2011, 97-102.

[11] A.P. Singh, and S. Kumar, Application of tannins in industry, Tannins- structural properties, biological properties and current knowledge, Alfredo Aires, *IntechOpen*, 2019, DOI: 10.5772/intechopen.85984.

[12] Z.S.S. Alhashemi and M.A. Hossan, Biological activities of different neem leaf crude extracts used locally in ayurvedic machine, Pacific Science Review A: *Natural Science and Engineering*, 2016, 128-131.

[13] D. Cox-Georgian, N. Ramadoss, C. Dona, C. Basu, Therapeutic medicinal uses of terpenes, *Medicinal Plants*, 2019, 333-359.

[14] Z. Nassar, A. Aisha, A. Abdulmajid, The pharmacological properties of terpenoids from Sandoricum Koetjape, *WebmedCecntral Complementry Medicine*, 1(2), 2010, WMC001311.

[15] G. Wang, W. Tang, and R.R. Bidigare, Terpenoids as therapeutic drugs and pharmaceutical agents, *Natural Products*, 2005, 197-227.

[16] B. Singh, and R.A. Sharma, Plants terpenes: Defense responses, phylogenetic analysis, regulation and clinical applications, *Biotech*, 5, 2015, 129-151.

[17] N. Z. Mamadalieva, F. Hammann, M. Z. El Reaadi, Flavonoids in scutellania immaculate and s. ramosissima (Lamiaceae) and their biological activity, *Journal of Pharmacy and Pharmacology*, 63, 2011, 1346-1357.

[18] S. Kumar, and A.K. Pandey, Chemistry and biological activities of flavonoids: An overview, *The Science World Journal*, 2013, Article ID 162750.

[19] D. Kregiel, J. Berlowska, I. Witonska, H. Antolak, C. Proestos, M. Babic, L. Babil, and B. Zhang , Saponin based biological active surfactants from plants, 2017, *IntechOpen*, DOI: 10.5772/68062.

[20] G. Pandey, K. Verma, K.M. Singh, Evaluation of phytochemical antibacterial and free radical scavenging properties of Azadirachta indica (neem) leaves, *International Journal of Pharmacy and pharmacology Science*, 6, 2014, 0975-1491.

[21] A. Okewale, F. Omoruwuo, Neem leaf extract as a corrosion inhibitor on mild steel in acidic solution, *International Journal of Engineering Research in Africa*, 35, 2018.

[22] F. Hassan, A. Umer, M. Sabri, A. Sarosh, Use of Neem leaf for oil removal from waste water, *Journal of Engineering and Applied Science*, 23, 2018, 86-92.

[23] J.B. Harborne, *Phytochemical methods*, London, Chapman and Hall Limited, 1973, 49-188.

[24] B.A. Boham, M.R. Koupai-Abyazani, Flavonoids and condensed tannins from leaves of Hawaiian vaccinium vaticulatum and V. calycinium, *Pacific Science*, 48, 1974, 458-463.

[25] B.O. Obadoni, P.O. Ochuko, Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta states of Nigeria, *Global Journal of Pure and Applied Science*, 8, 2001, 203-208.

[26] D. Pearson, *Chemical analysis of food*, 7th edition, Henry Edward company, Churchchill Livingstone, London, (1976) 218-336.

[27] N.M. Ferguson, *A text book of Pharmacognosy*, Mac Milan Company, New Delhi, (1956) 191.