

Study on the Relation Patterns of Bitter Leaf Juice Phytochemicals at Varying Temperature

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Abstract -Bitter leaf juice is known to have nutritional and therapeutic benefit which depends on its constituents for its efficiency when consumed. In Nigeria, bitter leaf juice is prepared and consumed at room temperature and elevated temperatures for treatment of malaria, reducing blood sugar levels, as anti-oxidants, anti-bacterial and other numerous medicinal and pharmaceutical properties. The argument of what temperature is suitable for the juice consumption and its efficacy always arises. It becomes necessary to investigate how varying temperature affects the physiochemical and phytochemical properties of the juice, its bitter taste and the relationship patterns in which this variation takes place. This project work looks at the relationship patterns between the phytochemical properties of bitter leaf juice at varying temperature and their probably benefit. Laboratory quantitative analysis of some physical properties (pH, specific gravity and conductivity) and phytochemical components (alkaloids, tannins, saponnins, flavonoids, terpenoids and phenols) were carried out for bitter leaf juice samples heated within temperatures 40°C, 60°C, 80°C and 100°C and then compared to the quantitative analysis of the bitter leaf extract sample at room temperature (25°C). The result showed a significant reduction in the phytochemical constituents where alkaloids and phenols reduces linearly with increasing temperature, tannins, saponnins, flavonoids and terpenoids reduces with increasing temperature in polynomial form of second order. The pH and specific gravity also decreases as temperature increases in polynomial form of second order while the conductivity increases with increasing temperature. These change showed that the medicinal and pharmaceutical properties of the juice and bitter taste decreases with increasing heat. The bitter leaf juice becomes, more acidic, less dense than water and more conducting at high temperatures. Thus, the juice prepared at 25°C is best suited for nutritional and therapeutic benefit and if heat is to be applied to bitter leaf juice, the temperature 40°C is recommended since at this temperature, the composition of the phytochemical and physiochemical properties is not much different from that at 25°C.

Keywords - Bitter Leaf Juice, Temperature, Physiochemical, Phytochemical Properties, Relation Pattern.

I. INTRODUCTION

Bitter leaf also known as *Vernonia amygdalina* has been widely used for both nutritional and therapeutic purposes such as the treatment and prevention of certain ailments such as malaria, typhoid, digestion problems, diabetes, infections, etc. due to its phytochemicals (biological active components). It is also widely known for its nutritional and detoxifying purposes in human body. The leaves are eaten as vegetables mostly used in cooking a popular dish called bitter leaf soup in Nigeria and the juice which is extracted from the leaves is either taken fresh or boiled. It is common to boil the bitter leaf juice in order to reduce the bitter taste caused by alkaloids, tannin, saponnins and glycosides present in the leaf (bonsi et al., 1995, Ologunde et al., 1992,

and Butley and Baily, 1973). Although most times it is difficult to detect the level of difference in taste, the effect of heating on other physiochemical properties of the extract is uncertain since there is no concrete knowledge of the changes in the physical and chemical properties of the juice that occurs when the juice is heated.

Consequently, research has focused on the components of bitter leaf juice and its relation to the significant properties of bitter leaf towards its nutritional and therapeutic value. Bitter leaf extract contains fat, protein and fibre which are necessary for human growth and maintenance (Ugwoke, *Nzekwe* and *Ameh*, 2010, Aliero and Abdulahi, 2009). Proteins are needed for body building and tissue repairs, fibres reduce cholesterol in blood, vitamins

and minerals perform hundreds of roles in the body, amongst their numerous functions is human maintenance and development (Jim and Stewart, 2017). Similarly, in a comparative study it was affirmed that bitter leaf contains high amounts of crude protein, fibre, vitamins and minerals (Atangwo et al, 2009), which are important in human diet.

Apart from bitter leaf being a necessary addition to the diet for human health maintenance it also performs roles in the treatment and ablation of certain diseases and illness through its resistance ability to bacteria, diabetes, malaria, parasites, inflammation, helminths, oxidation, etc. (Atangwo et al, 2010, Yeap et al, 2010, Aghogidi et al, 2013). Research has shown that bitter leaf possesses these resistance due to certain components such as alkaloids, saponins, tannins, steroids, flavonoids, etc. (Bergman, 2000., Ogundare., 2011, Offor, 2014).

Research works have also been intensive on the conservancy of the important constituents and the efficiency of bitter leaf juice. Since the constituents are of high importance it is necessary to preserve it so as to maintain its efficacy when used. This was enlightened in an analytical study where it was supported that the extraction method of bitter leaf juice and some other factors may affect its efficiency. (Udochukwu et al, 2015). In line with this, it was reported that the extraction and preservation methods of bitter leaf juice affects its loss of Vitamin C (Ejoh et al, 2005).

The extraction of bitter leaf juice is done using several methods alternating from hand washing, stone grinding, solvent extraction, blending, etc. of the leaves. One of the aims during extraction of the juice is to avoid loss of important components to the minimum level. In a study, the method of squeeze washing the leaves was proved to be the best extraction method since there was minimum loss of vitamins (Ejoh et al, 2005).

Lastly none of these studies have shown the effect of change in temperature on the physiochemical and phytochemical properties of bitter leaf juice as temperature is a strong dependent variable that may or may not affect certain properties of bitter leaf juice which will sequentially affects the efficiency of the juice. This research work focuses on the effect of varying temperature on some physiochemical and phytochemical properties of the bitter leaf juice and their relationship patterns which indicates the forms this variation takes place and also determines the usefulness and medicinal efficacy of the leaf and its bitter taste.

II. MATERIALS AND METHOD

The following materials and equipment were used for the research work: Powdered Bitter leaf sample, Distilled water, Ethanol (99%), Acetic acid solution, Ammonia solution, Ammonia hydroxide, Tannic acid (standard), Folin-Denis Reagent, Saturated sodium carbonate solution, Methanol, Dimethyl ether, N-butanol, Sodium chloride solution, Petroleum Ether, Amyl alcohol, 100ml conical flask, 250ml separating funnel, 50ml volumetric flasks, Boiling tubes Centrifuge, Conductivity probe, drying oven ($\leq 350^{\circ}\text{C}$), Electronic weighing balance, Hot plate, Incubator, pH Meter (-2 – 14), Plastic beaker (50ml), Porcelain crucible, Specific gravity bottle, UV/VIS Spectrophotometer, Whatman No. 42 Filter paper.

A. Method

1. Preparation of Samples

Bitter leaf was purchased at Amassoma market in Amassoma, Southern Ijaw, Bayelsa state in Nigeria. The leaves were sundried for 3 days and then grinded to fine powder.

2. Determination of Physical Constants

50ml of distilled water was added to 10 grams of grinded leaves and the leaves were extracted by filtering the mixture. The resulting green solution was further filtered with a straining cloth. Additional 20ml of distilled water was then added for the final extraction,

3. pH Measurement

20ml of the juice extract was placed in a plastic beaker and the electrode of a pH meter was dipped into the solution and a steady read out was taken as the pH of the extract. The solution was then heated to 40°C and the pH was measured again. This was repeated for the solution after heating to 60°C , 80°C and 100°C .

4. Conductivity Measurement

A conductivity probe was dipped into 20ml of extract and the steady read out was recorded as the conductivity of the solution. The solution was heated to 40°C and the conductivity was recorded. This was done for the solution at temperature 60°C , 80°C and 100°C .

5. Specific Gravity Measurement

A 50ml specific gravity bottle was weighed empty and the weight noted.

The bottle was filled to the brim with the extract solution and the cap placed on the mouth of the bottle to allow the

excess to flow out. The bottle was wiped dry and replaced in the balance and weight was recorded.

The specific gravity of the liquid was calculated from the formula shown in (1).

$$S. \text{ gravity} = \frac{\text{Mass of extracts}}{\text{Vol. of spec. gravity bottle}} \quad (1)$$

This process was repeated for the extract solution heated at temperatures of 40°C, 80°C and 100°C.

B. Determination of Phytochemical Constituents

1. Determination of Alkaloids

The gravimetric method used by Harbone was adopted (Harbone, 1973). The following steps were followed (1) 5 grams of grinded sample was dispersed in 50ml of 10% acetic acid solution in ethanol. (2) The mixture was shaken properly and allowed to stand for 4 hours and then filtered. (3) The filtrate was evaporated to about $\frac{1}{4}$ of its original volume. (4) Ammonia solution was added drop wise into the solution to precipitate the alkaloids out of the solution (5) the precipitates were filtered in a pre-weighed filter paper and was washed further with 1% ammonium hydroxide. (6) The precipitate was then dried along with the filter paper in the oven at 60°C for about 1 hour. (7) The amount of alkaloids was obtained by weight difference and expressed as a percentage of the sample weight analysed as shown in equation (2).

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W} \times 100 \quad (2)$$

Where; W = Weight of sample, W_1 = Weight of filter paper, W_2 = Weight of filter paper + precipitate

Steps 1 and 2 were repeated but this time the filtrate was heated to temperature of 40°C and steps 3 – 7 were repeated. This process was repeated for extracts heated to 60°C, 80°C and 100°C.

2. Determination of Tannins

The Folin-Denis spectrophotometric method was used as described by Pearson (Pearson, 1976). The procedure is as follows; (1) 1 gram of powdered sample was weighted into a tube and 10ml of distilled water was added. (2) The mixture was shaken well and left to stand for 30 minutes. (3) This was then centrifuged for 10 minutes at 3000rpm. (4) 2.5ml of the supernatant extract was dispersed into a 50ml volumetric flask. (5) Similarly, 2.5ml of standard tannic acid solution was dispersed into another 50ml volumetric flask.

(6) 1ml aliquots of Folin-Denis reagent was measured into the various volumetric flasks followed by 2.5ml aliquots of saturated sodium carbonate solutions. (7) The mixtures were then diluted to the 50ml marks in each flask. (8) The flask were incubated for 90 minutes at room temperature (9) The UV/VIS spectrophotometer was set at 250nm (Jenway 6500) after the instrument was zeroed. (10) The tannin content was obtained from equation (3).

$$\% \text{ Tannins} = \frac{A_n}{A_s} \times C \times \frac{100}{W} \times \frac{V_f}{V_a} \quad (3)$$

Where; W = weight of sample, A_n = absorbance of test sample, A_s = absorbance of standard sample, C = concentration of standard tannin solution, V_f = total volume of extract, V_a = volume of extract analysed.

Steps 1 – 3 were repeated. The supernatant extract was then heated to a temperature of 40°C. Then steps 4 – 10 were repeated to get tannin content. This was carried out for the supernatant extract heated to temperatures 60°C, 80°C and 100°C.

3. Determination of Flavonoids

The Bohm and Kocipal-Abyazan method was used (Bohm and Kocipal-Abyazan, 1994). Briefly the following steps were followed; (1) 10 grams of powdered sample was repeatedly extracted with 100ml aliquots of 80% aqueous methanol. (2) The extract was filtered through Whatman No.42 filter paper. (3) The filtrate obtained was transferred into a porcelain crucible and evaporated to dryness over a water bath; and it weighed up to a constant weight. (4) The percentage content of flavonoids was then calculated using equation 4.

$$\% \text{ Flavonoid} = \frac{W_p}{W_s} \times 100 \quad (4)$$

Where; W_p = weight of precipitate, W_s = weight of sample

Step 1 and 2 were repeated. The filtrate was then heated to a temperature of 40°C; steps 3 and 4 were repeated to get the flavonoid content of extract at 40°C. These processes were repeated for the filtrate at temperatures 60°C, 80°C and 100°C.

4. Determination of Saponins

The technique applied by Obadoni and Ochuka (Obadoni and Ochuka, 2001) was adopted. The following steps were used. (1) 10 grams of powdered sample was weighed into 100ml conical flask and 50ml of 20% aqueous ethanol was added. (2) The sample was placed on a water

bath at 55°C with continuous stirring for 4 hours and the mixture was then filtered. (3) The residue was also re-extracted with another aliquot of aqueous ethanol. (4) Both extracts were combined and concentrated down to 40ml over a water bath kept at 90°C. (5) The resulting concentrate was transferred into a 250ml separating funnel. (6) 10ml aliquots of dimethyl ether was added and shaken vigorously. (7) Two layers were formed in the funnel, which the aqueous layer was recovered, and the ether layer was discarded. (8) The aqueous layer was then re-extracted with 30ml of n-butanol. (9) The combined layer with n-butanol was washed with aqueous sodium chloride solution twice. (10) The remaining solution was then placed in water bath and heated to dryness. (11) The residue obtained was dried in an oven to a constant weight and the saponins content percentage was calculated from equation 5.

$$\% \text{ Saponnin} = \frac{W_r}{W_s} \times 100 \quad (5)$$

Where; W_r = weight of residue, W_s = sample weight

Steps 1 – 3 were repeated. Both extracts were combined and then heated to temperature 40°C.

The extracts were then placed over a water bath and steps 4 – 11 were repeated. This was repeatedly done for the extract heated to temperatures 60°C, 80°C and 100°C.

5. Determination of Terpenoids

Ferguson's method was adopted (Ferguson, 1956) Procedure; (1) 10grams of powdered sample was taken and soaked in methanol for 24 hours and was filtered through cotton wool. (2) The cotton wool was wasted with an extra 10ml of fresh methanol. (3) The filtrate was transferred into 250ml separating funnel. (4) The filtrate was then extracted twice with 20ml aliquots of ether. (5) The ether extract was evaporated till dryness over a water bath and weighed up to obtain a constant weight. (6) The terpenoids content was calculated as a percentage from equation 6.

$$\% \text{ Terpenoids} = \frac{W_r}{W_s} \times 100 \quad (6)$$

Where; W_r = weight of residue, W_s = sample weight

Steps 1 and 2, were repeated and the filtrate heated to 40°C, followed by steps 3 – 5. These processes were repeated for filtrate extract at temperatures 60°C, 80°C and 100°C.

6. Determination of Phenols

Phenols content was determined using spectrophotometric method. The following steps were followed; (1) 5 grams of the powdered samples were defatted by soaking in ether for 1 hour and then filtered. (2) The filtrate was then boiled in 50ml of ether for 15minutes to extract the phenolic fractions. (3) 5ml of the extract was transferred into a 50ml volumetric flask and 10ml of distilled water. (4) A further 5ml of fresh amyl alcohol was added and the mixture was made up to mark with distilled water. (5) The flask was left to stand for 30minutes for colour formation. (6) The spectrophotometer was set at 505nm. (7) The phenolic content was obtained.

Steps 1 and 2 were repeated. The extract was then heated to 40°C and steps 5 – 7 repeated. These processes were repeated for extract at temperatures 60°C, 80°C and 100°C.

III. RESULTS AND DISCUSSION

Table 1 shows the result for the quantitative phytochemical analysis of bitter leaf samples analysed after taking the average of triplicate values.

Table 1. Phytochemical Constants in Relation to Temperature Variation

Temp	%AKL	% TAN	% FLAV	% SAP	% TERP	%PHE
25	14.91	6.25	0.34	2.15	1.30	0.19
40	13.59	5.88	0.30	2.12	1.26	0.17
60	11.40	4.38	0.28	2.00	1.21	0.14
80	9.36	2.80	0.23	1.70	1.10	0.11
100	7.57	1.45	0.17	1.46	0.80	0.09

From the table, the phytochemicals (alkaloids, tannins, flavonoids, saponins, terpenoids and phenols) reduces as temperature increases. This trend showed that the medicinal efficacy of the bitter leaf juice due to the presence of alkaloids, tannins, saponins and flavonoids (Abosi, et al, 2003, Ogundare, 2011, Offor, 2014, Mensah et al, 2008), decreases with increased heating. This result is corroborated with the results in Fig. 1, 2, 3, 4, 5 and 6 for alkaloids, tannin, flavonoids, saponins, terpenoids and phenol respectively.

Fig.1 showed that alkaloids which contributes to the bitter taste, anti-bacteria and anti-cancer properties of bitter leaf (Ebenezer, 2011., Saalu et al, 2013) decreases with increasing temperature linearly with relation pattern shown in equation 7 and has reliability prediction R^2 of 0.9988

approximately 1. As indicated by the dotted plot in the figure. This showed that the model perfectly predicted the behaviour of alkaloids with increasing temperature.

$$\text{Alk} = -0.0996T + 17.44 \quad (7)$$

Fig. 2, 3, 4, 5, showed that tannins, flavonoids, saponins and terpenoids respectively decreases with increasing temperature in a polynomial relation pattern of second order. As shown in equation (8), (9), (10) and (11) with R^2 of 0.992 for tannins, $R^2 = 0.9874$, for flavonoids, $R^2 = 0.9896$ for saponins and $R^2 = 0.982$ for terpenoids. The R^2 which can be easily approximated as 1 showed that the relation patterns perfectly predicts the change in tannins, flavonoids, saponins and terpenoids respectively.

It is known that Tannins possess antibacterial, antimicrobial, anti-oxidative and anti-carcinogenic properties, as well as reducing blood pressure and accelerating blood clot (Chung et al, 1998).

Flavonoid has antioxidant activity, anti-virus and anti-inflammatory properties (Iwawela et al, 2005; Eleazu et al, 2012). Saponins also contributes to the bitter taste of bitter leaf and exhibits antibiotic activity as well as aiding wounds to heal quickly by enhancing coagulation of red blood (Francois et al, 2017; Rao et al, 1995).

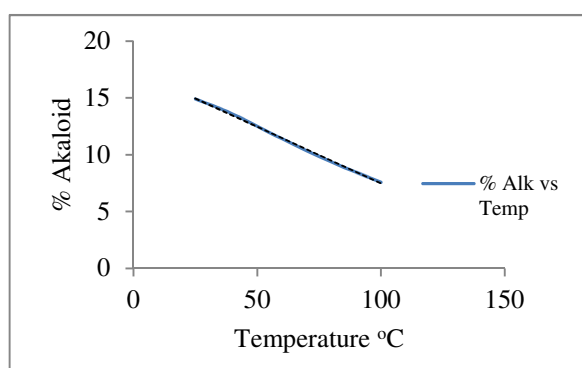


Fig. 1 Percentage Alkaloids against temperature

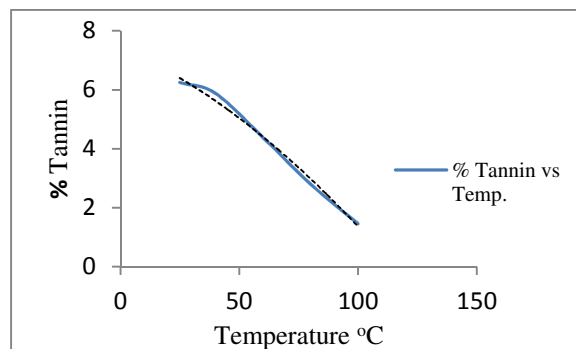


Fig. 2 Percentage Tannin against temperature

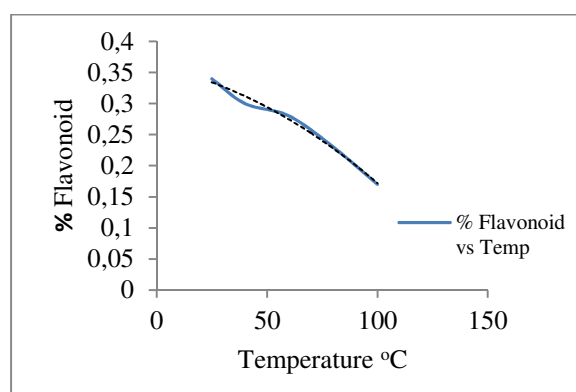


Fig. 3 Percentage Flavonoids against temperature

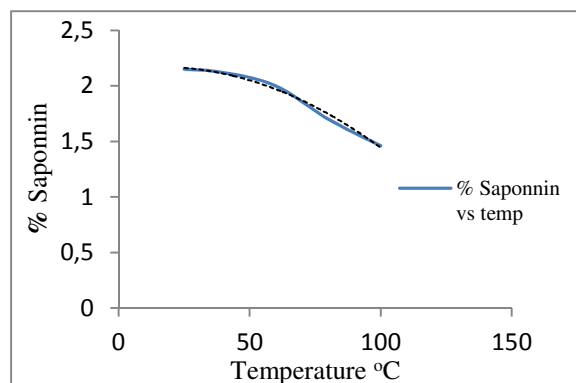


Fig. 4 Percentage Saponnins against temperature

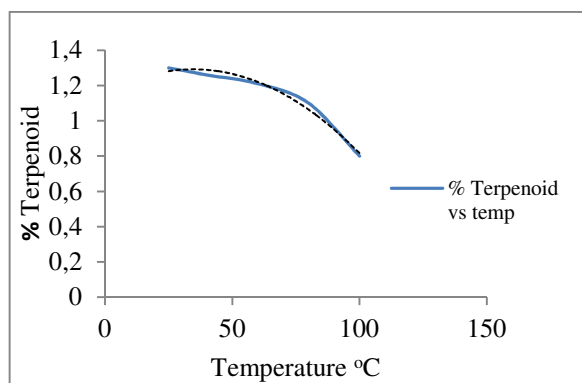


Fig. 5 Percentage Terpenoids against temperature

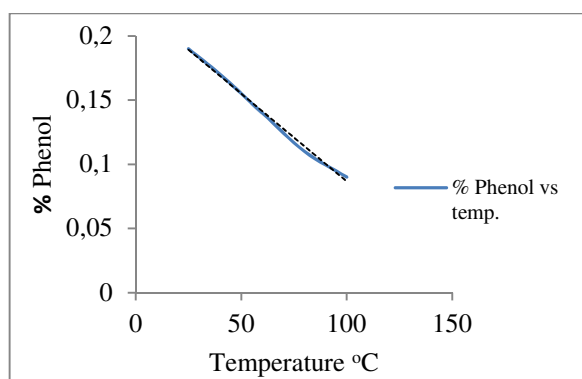


Fig. 6 Percentage Phenols against temperature

Fig. 6 showed that phenol decreases with increasing temperature linearly with relation pattern in equation 12 and $R^2 = 0.9954$.

$$\text{Tan.} = -0.0003T^2 - 0.0352x + 7.4361 \quad (8)$$

$$\text{Flav.} = -1e^{-05}T^2 - 0.0008x + 0.3602 \quad (9)$$

$$\text{Sap.} = -0.0001T^2 - 0.0032x + 2.1447 \quad (10)$$

$$\text{Ter.} = -0.0001T^2 - 0.0077T + 1.1573 \quad (11)$$

$$\text{Phe.} = -0.0014T + 0.2234 \quad (12)$$

Phenols have been associated with the inhibition of cancer and also possess antioxidant behaviour (Iwalewa et al., 2005). These results indicate the medicinal properties of the phytochemicals of bitter decreases with temperature.

Table 2 shows the result of the physical constants analysis of the bitter leaf samples analysed.

Table. 2 Physical Constants in Relation to Temperature Variation

Temp (°C)	pH	Conductivity (μS/cm)
Spec. gravity		
25	6.82	1728
40	6.76	1730
60	6.54	1735
80	6.20	1745
100	5.70	1760

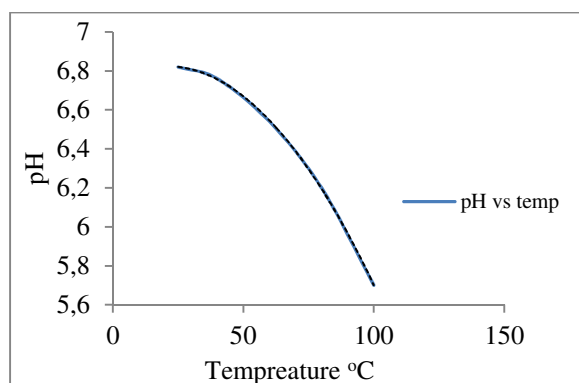


Fig.7 Effect of temperature on pH of Bitter leaf juice

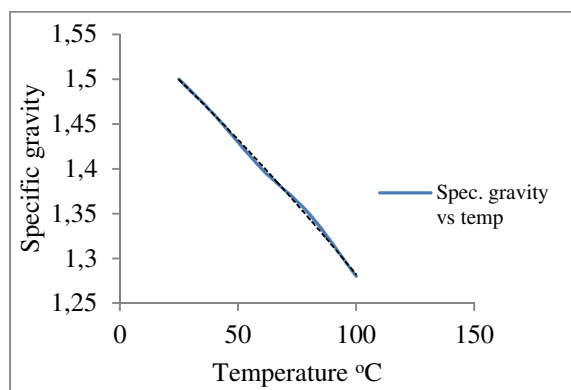


Fig.8 Effect of temperature on specific gravity of Bitter leaf juice

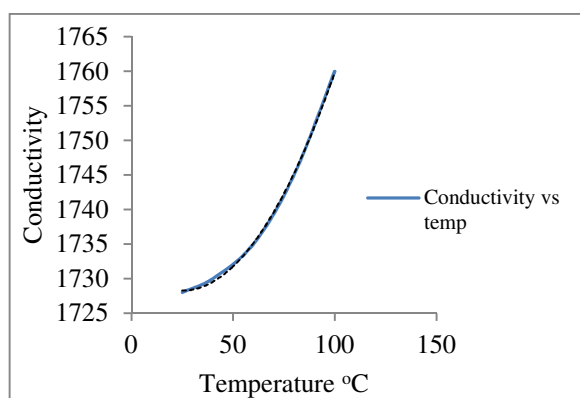


Fig.9 Effect of temperature on conductivity of Bitter leaf juice

The pH and specific gravity of the bitter leaf extract reduces as temperature increases in polynomial relation pattern of second degree as shown in equation 13. This shows that bitter leaf become more acidic as temperature increases. Thus if bitter leaf is to be taken at elevated temperatures it should be at the right conditions and environment. The reduction in the specific gravity of the bitter leaf extract as temperature increases in equation 14 shows that bitter leaf juice becomes less dense than water with increasing temperature in polynomial form. Also the conductivity of the juice increases with increasing temperature in polynomial relation pattern of second order too shown in equation 15. This shows that the bitter leaf extract juice becomes more conductive to electricity with increasing temperature.

$$\text{pH} = -0.0002T^2 - 0.0072T + 6.7513 \quad (13)$$

$$\text{Sp. g.} = -5e^{-06}T^2 - 0.0023T + 1.5604 \quad (14)$$

$$\text{Cond.} = 0.0056T^2 - 0.2825T + 1731.8 \quad (15)$$

IV. CONCLUSION

This research work has shown bioactive components (alkaloids, tannins, flavonoids, saponins, terpenoids and phenols) present in the bitter leaf juice extract is affected with increasing temperature. Tannins, flavonoids, saponins and terpenoids decreases in polynomial form of second order with increasing temperature while alkaloids and phenols decrease linearly. The pH, specific gravity of the extract also had second order polynomial relation patterns as they decreases with increasing temperature while the conductivity increases with increasing temperature. This

change in turn affects the medicinal and pharmaceutical properties of the bitter leaf juice. Thus, taking bitter leaf juice prepared at room temperature (25°C) is best for nutritional and medicinal applications. If heat is to be applied, 40°C is the best temperature for heating the juice before consumption. This is because at this temperature, there is no significant loss in the percentage composition of the phytochemical and physiochemical properties as compared to that at 25°C.

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