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Analysis of Solids from Crude Leaf Extracts of *Gongronema Latifolium*

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Abstract:
This research work was conducted to analyze solids from crude leaf extracts of Gongronema latifolium often employed in treating diabetics using the thin layer chromatography and ultraviolet spectroscopy methods. Gongronema latifolium (GL) fresh leaves were dried, pulverized and the liquid soluble portion gotten by soxhlet extraction using ethyl acetate and ethanol as solvents. Thin layer chromatography was done on both extracts, which gave good separation. Again, some of the fresh leaves were expressed and extract of the soluble portion gotten. This portion was separated into the acidic, basic and neutral components. Three of them gave crystals on evaporation, these crystals were further purified and analyzed using ultraviolet spectroscopy and their absorption spectrum interpreted. From thin layer chromatography, colors of green, yellow and black were seen, showing the presence of colored compounds in the leave. Extracts of the soluble portion yielded 12.8g acidic crystals, 16.0g basic crystals and 7.5g neutral crystals. Ultraviolet spectroscopy was done on the crystals, the results showed that the absorption spectrum for the acidic component of the solid extracts can be related to the absorption bands of three organic acidic compounds. That for the basic portion can be related to the absorption bands of two organic basic compounds which are both derivatives of hydrogen cyanamide. While that for the neutral portion can be related to the absorption bands of two organic neutral compounds. These organic components are believed to be the active constituents in the leaves of Gongronema latifolium leading to its use for treatment of diabetes.

Keywords: *Gongronema latifolium, extraction, thin layer chromatography, ultraviolet spectroscopy.*

1. Introduction

Gongronema latifolium (GL) called utazi leaf in South Eastern part of Nigeria is an edible green leaf vegetable with climbing stem. The leaf is heart-shaped with reticulate venation and smooth margin, has no seeds, the tree has to be distributed through cutting. It is a very homely plant and flourishes where it grows. It produces large mass of foliage and is drought tolerant. Its bitterness is whether cooked or raw. GL leaf is a tropical plant; it flourishes widely in the bush and forest. It is also cultivated in gardens. It is found in Nigeria, Ghana, India, China and some hot countries. It could be consumed as leafy vegetables, it is consumed because of its characteristic bitter taste which stimulates appetite. Health wise, it has been used by herbalists in past years for the treatment of so many diseases like fever, diarrhea and ulcers (Akuodo *et al.*, 2010). It is also a rich source of iron. So many studies have been carried out on GL (Nwinyi *et al.*, 2008, Gamaniel *et al.*, 1996, Obinna *et al.*, 2008, Schneider *et al.*, 1993, Oshodi *et al.*, 2004) Essien et al. researched on the use of GL for the treatment of fowl cough. Several authors have researched on the use of GL extract for treatment of diabetes in rats (Nwanjo *et al.*, 2006, Ugochukwu *et al.*, 2003, Edet *et al.*, 2004, Antai *et al.*, 2009) but to the best of our knowledge the active component responsible for this has not been analyzed. (Eleyinmi, 2007) studied the chemical and antibacterial activity of GL using standard methods. It was found that it contains metals like K, Na, Ca, P and Co, amino acid, aspartic acid, glutamic acid, palmitic acid and glycine. (Morebise *et al.*, 2008) showed that the phytochemical analysis of GL contains alkaloids, glycosides, phenols and flavonoids. However, phytochemicals are chemical compounds which may affect health but are not yet established as essential nutrients. This study focuses on the analysis of solids from crude leaf extracts of *Gongronema latifolium* for diabetic treatments using the thin layer chromatography and ultraviolet spectroscopy methods.

2. Material and Methods

2.1. Plant Material

Fresh leaves of *Gongronema latifolia* were gotten from Owerri in Imo State, Nigeria. Samples of the leaves were identified in the Crop Science Department of School of Agriculture and Agric Technology, SAAT in Federal University of Technology Owerri, Imo State, Nigeria. The sample was then washed with distilled water and rinsed again, it was weighted with an electronic machine and the weight noted. The fresh leaves were then air dried under shade for 8 days and weighed twice to ensure constant weights. They were pulverized using an electric blender (Qlink, Germany design) and wrapped with filter papers.

2.2. Extraction

The soxhlet apparatus is made up of a system of continuous extraction from a solid by a hot solvent. Two solvents were used consecutively; ethyl acetate and ethanol. The sample prepared was put in the porous thimble and placed in the soxhlet apparatus. The apparatus is then fitted to a round-bottomed flask containing 200 mL of ethyl acetate and held tight with a clamp, some boiling chips were also added to the round-bottomed flask to reduce bombing. The apparatus was then fitted to a condenser. The solvent was heated gently for 6 hours using a water bath. The solvent generated vapor, which passes up through the tube and is condensed by the condenser. The condensed vapor falls into the thimble as liquid, it slowly fills the body of the soxhlet, saturating the sample and extracting the soluble liquid components of the solid, this gives the solvent in the thimble a greenish coloration. When the solvent reaches the top of the tube, it siphons over into the round-bottomed flask. The process is repeated automatically until complete extraction is observed. The extract was recovered from the various solvents by simple distillation method. This was done by pouring the product of extraction into another round-bottomed flask and connected to a distillation set up. It was heated slowly in a water bath at 100^{o}C. The solvent evaporated, passed through the distillation column and was recovered, while the extracted component was left in the round-bottomed flask. This process was also repeated using ethanol as a solvent.

2.3. Thin Layer Chromatography (TLC)

Test was conducted using ethyl acetate and hexane as solvents. 5 mL of ethyl acetate was combined with 4 mL of hexane and used as the solvent system. This gave good separation because of the difference in the polarity of the solvents. While silica gel coated on a plastic surface was used as the TLC plate, two spots of the samples were made on the plate using a small capillary tube and then it was allowed to dry. The separated spots were visible likewise the colors, nevertheless the plate was still placed in a beaker containing few iodine crystals so that undeveloped spot would be noticed but there was no difference so the plate was brought out and the R.F values calculated.

2.4. Ultraviolet Spectroscopy (UV)

2.4.1. Extraction of the Solid Components of GL for UV Analysis

500g of GL leaves were washed, shaken free of water droplets and then expressed. 200 mL of the extract was separated out as sample for the extraction of the solid components of the leaves, this was poured into a seperatory funnel and clamped, 2 M NaOH, 2 M HCl and ethyl acetate were used to extract the acidic, basic and neutral components, these were washed with brine solution and dried with anhydrous magnesium sulphate. The dried portion, were evaporated to get the acidic, basic and neutral portions which were all recovered as white crystals. Each of the crystals were purified by dissolution in a suitable solvent. This was done by heating gently in a water bath to ensure completion. The solutions were then filtered hot, the undissolved impurities were deposited on the filter paper while the recovered solution was evaporated to get back the crystals. The purified crystals were found to be brighter in color and more coarsely dispersed. After purification, the crystals were quickly transferred back into the bottles, covered tightly and stored back on the shelf.

2.4.2. UV Analysis

Ultraviolet spectroscopic analysis of these solid components were then carried out using 0.5 g in the appropriate solvent, the intensity of radiation at each frequency was then analyzed by a suitable detector and the graph of the results plotted.

3. Result and Discussion

3.1. Thin Layer Chromatography

Thin layer chromatography is used to know how many components are in a mixture, it is also used to support the identity of a compound in a mixture. These components differ in their solubility and strength of adsorption to the adsorbent, also, the strength with which they bind to the adsorbent depends on the strength of the dipole-dipole interaction between the adsorbent and the separated components. The compounds with larger R_f are less polar because they interact less strongly with the polar adsorbent on the TLC plate as has been studied by some authors (Obomanu *et al.,* 2005, Dashe *et al.,* 1998).

% Recovery for ethyl acetate after extraction was 20.5%
% Recovery for ethanol after extraction was 15.1%

Retention Factor $(Rf) = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$

Table (1) illustrates the extract from GL leaf with ethyl acetate, it showed the separation of three colors; green, black, yellow. But five different sports were seen at various distances. This shows that the extract has five different components three of them has the same color with different shades showing that they maybe the same compound but at different concentrations. But the R_f values of the three components differs which means that they could be different compounds having the same color since the R_f value is a characteristic of a compound, and can be used to identify compound in an unknown mixture. Two of the spots had different colors which are black and yellow meaning that they are completely different compounds. They both also had different R_f values which tells also that they are different compounds.

Colors of spots	Distance moved by spots(cm)	R_f values
Green	1.0	0.15
Black	1.3	0.19
Yellow	3.0	0.44
Light green	3.5	0.52
Dark green	4.2	0.62

Table 1: Extract from GL leaf with ethyl acetate

Distance travelled by solvent front = 6.8cm

Table (2) shows the extract from GL leaf with ethanol. This shows the separation of four spots into three colors. This indicates that this extract contains four different components since their R_f values also differs. From the results, it showed that ethyl acetate extracted more components more than ethanol and that ethanol extracted the components that ethyl acetate was not able to extract. We observed also, that the black color observed in both chromatographic separations disappeared slowing on drying changing into a green color.

Colors of spots	Distance moved by spots(cm)	R_f values
Green	1.60	0.24
Black	2.20	0.33
Green	2.65	0.39
Yellow	2.95	0.43

Table 2: Extract from GL leaf with ethanol

Distance travelled by solvent front = 6.8cm

3.2 Ultraviolet Spectroscopy Results

Ultraviolet spectrometers measures absorption of light in the visible and near ultraviolet region, that is, in the 200-750 nm range. This light is of higher frequency and greater energy than infrared light and when it is absorbed by a molecule, it produces changes that require greater energy that is, changes in electronic states; it does so by absorbing radiation at definite frequencies. This suggests that molecules can possess only discrete energies, not an arbitrary energy. (Peter and Julio, 2010, Morrison and Boyd, 2001). UV has been found to aid the identification of some many organic compounds (Nakagowa *et al.*, 1999, Udoha *et al.*, 2005, William, 1979). Organic compounds in plants have been discovered by several authors to bring about anti-diabetic activities of the plant. (Steve, 2009, Cam *et al.*, 1993, Liao *et al.*, 2007) These UV results shows some organic compounds in GL leaf which are believed to be responsible for the anti-diabetic activity of the leaves of the plant.

% Recovery of the basic component of GL leaves 25.0%
% Recovery of acidic component of GL leaves 40.0%
% Recovery of the neutral component of GL leaves 35.0%

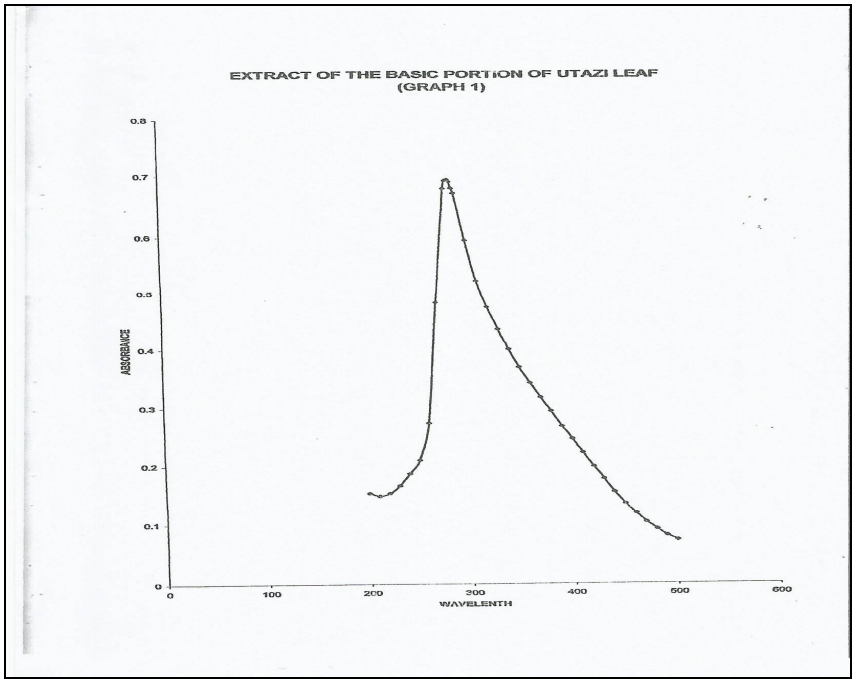

Figure 1: shows the absorption spectrum for the basic component of the solid extracts

From the spectrum, highest peak was seen at 280nm but the absorption band is within the ranges of 25nm and 380nm. This can be related to the absorption bands of some organic basic compounds which are:

1. Phenylcyanamide at 280nm
2. N – methylphenylcyanamide at 280nm

Which are both derivatives of hydrogen cyanamide.

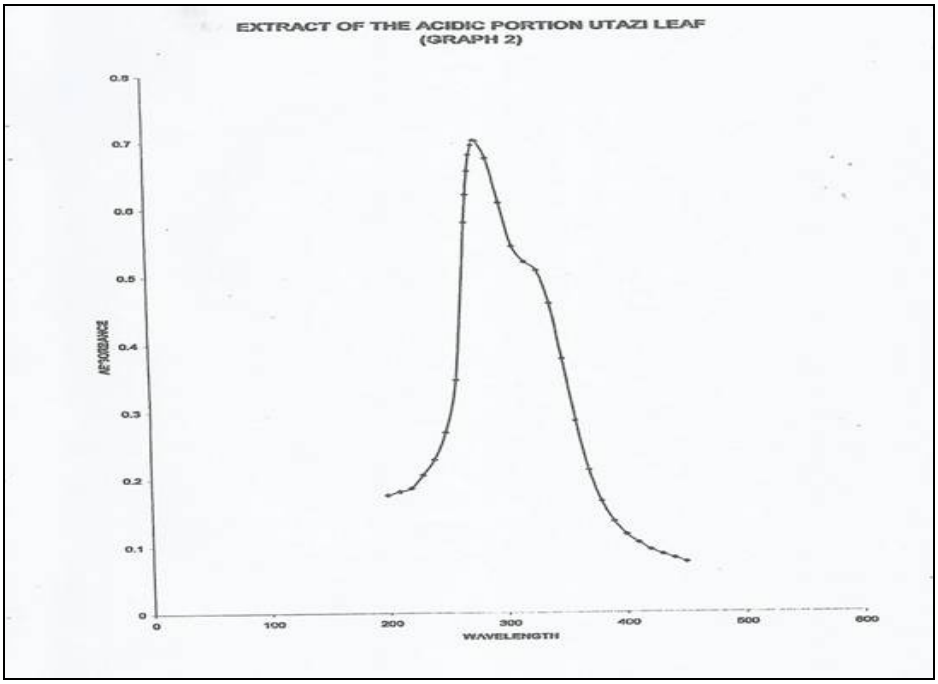

Figure 2: shows the absorption spectrum for the Acidic component of the solid extracts

From the spectrum, highest peak was seen at 289nm and with a minor peak at 330nm. The absorption band is within the ranges of 260nm and 350nm. This can be related to the absorption bands of some organic acidic compounds which are

1. Resorcinol at 277nm
2. Nitrobenzene at 270nm
3. Stilbene (cis) at 280

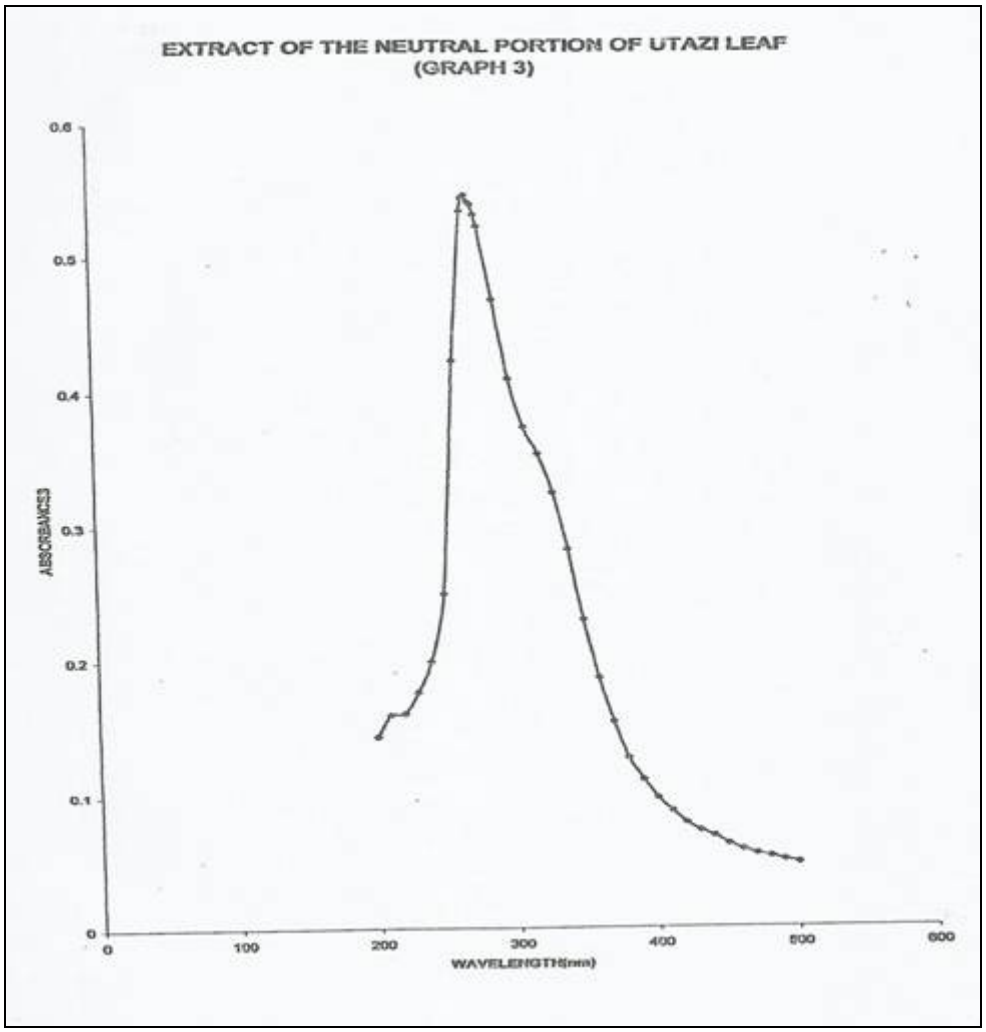

Figure 3: shows the absorption spectrum for the neutral (component) of the solid extract

From the spectrum, highest peak was seen at 270nm. The peak is within the ranges of 220nm and 360nm. This can be related to the absorption bands of some organic neutral compounds which are;

1. Croton aldehyde between 220nm and 32lnm.
2. Anisole at 269nm

4. Conclusion

GL leaf as mentioned earlier is given to lactating mothers and is believed to threat gestational diabetics. It is a vital plant that is very useful in the society and will continually be. From the TLC results, it showed that ethyl acetate extracted more components than ethanol, giving five components while ethanol gave four, implying that ethanol extracted the components that ethyl acetate was not able to extract. Ethyl acetate extract showed highest R_f value of 4.2 while ethanol extract showed highest R_f value of 2.95 indicating that compounds from ethyl acetate extract are less polar because they interact less strongly with the polar adsorbent on the TLC plate. The extraction of the solid components from GL leaf gave solid crystals for the acidic portion 16.0 g, basic portion 12.0 g and neutral 7.5 g portion, showing that the liquid expressed from the leaves contains solids which are organic. From the result of the UV analysis, the absorption spectrum for the acidic component of the solid extracts can be related to the absorption bands of three organic acidic compounds. That for the basic portion can be related to the absorption bands of two organic basic compounds which are both derivatives of hydrogen cyanamide. While that for the neutral portion can be related to the absorption bands of two organic neutral compounds. These solids are believed to be the active components in the leaves responsible for the treatment of diabetics. However, further research workis suggested to aid the isolation and findings regarding the structures of these compounds.

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