Proximate Composition and Functional Analysis of Some Polyherbal Formulations as Potential Botanical Candidates for Antidiabetic Screening: A Preliminary Study

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Abstract
Polyherbal formulations improve therapeutic action while lowering single herb concentration, thereby reducing possible side reactions. Proximate composition and functional properties of tomato, garlic, and carrot formulations were studied. Fresh samples of the botanicals were collected, identified and authenticated. Equal amount of each sample (500 g) was weighed, shed-dried and crushed into fine powdered particles. The proximate composition and functional properties of each formulation were determined according to a standard assay guideline. The result on proximate composition analysis revealed a significant (p < 0.05) higher moisture content of tomato and carrot formulations when compared to other formulations. The content of ash and crude protein were significantly (p < 0.05) higher in all garlic containing formulations. The garlic formulation was observed to have lower fat content and greater carbohydrate content than all other formulations (p< 0.05). However, when compared to single formulations, all mixed formulations had significantly higher crude fiber content. Furthermore, there was a significant (p < 0.05) variation in glucose adsorption capacity, oil adsorption capacity and water adsorption capacity among all the formulations. The outcome of this study have led us to conclude that employing safe combinations of these herbal formulations for the production of nutraceuticals is recommended.

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INTRODUCTION
The importance of human nutrition cannot be overstated because it is responsible for energy, tissue growth, tissue healing, disease immunity and other metabolic functions in the human system (Boadi et al., 2021). Proteins, lipids and oils, carbohydrates, fiber, vitamins, minerals, and water are all required by the human body for metabolic activities (Boadi et al., 2021), and they are very rich in common vegetables such as tomatoes, ginger, carrot, spinach, onion and cucumbers (Aderinola and Abaire, 2019). Proximate analysis is used to evaluate the relative amounts of protein, fat, water, ash and carbohydrate in each sample. In the food industry, some of these food components may be of interest for the purpose of product development, quality control or regulatory compliance (Thangaraj, 2015). Functional analysis, on the other hand, defines the behavior of ingredients during food preparation, as well as how the components affect the end product’s texture and flavour (Twinomuhwezi et al., 2020). Among the functional qualities are: oil adsorption capacity, water adsorption capacity, emulsion capacity, dextrinization, denaturation and gelatinization (Awuchi et al., 2019).

Tomato (Solanum lycopersicum L.), is a member of the Solanaceae family and it is a native of South America, particularly Peru and the Galapagos Islands (Matthew, 2011). It is a savory and edible fruit that is usually red in colour when ripe (Jafer, 2016). It is a perennial plant in its native habitat; however, it is also commonly planted as an annual in temperate climates (Ganesan et al., 2012). After potato, tomato is the world’s second most important vegetable (Dorais et al., 2008). Tomato is high in protein, has a small amount of fat and is also rich in vitamin B6, ascorbic acid, niacin and minerals that promote good health and serve as cofactors in enzymatic reactions (Garuba et al., 2018). Lycopene, one among the most effective natural antioxidants, is also found in the tomato fruit and it is responsible for its red colour (Ali et al., 2020). Lycopene, found primarily in cooked tomatoes, has been shown to prevent prostate cancer and improve the skin’s capacity to protect the body from damaging ultraviolet rays of sunlight (Garuba et al., 2018). Anticancer, anti-inflammatory, antidiabetic, anti-allergenic, anti-atherogenic, anti-thrombotic, antibacterial, antioxidant, vasodilator and cardioprotective properties are among the pharmacological activities of lycopene (Ali et al., 2020).

Garlic (Allium sativum), belongs to the Liliaceae family and it has a long tradition of medicinal use. According to some literatures, this was a medicinal herb discovered in the Avesta, a compilation of Zoroastrian Holy Scriptures gathered during the six century BC (Kshirsagar et al., 2018). It was utilized to treat a variety of ailments by the ancient Egyptians, Greeks, and Chinese. Furthermore, garlic’s components have been proven to have antiviral and anticarcinogenic properties (Mousavi et al., 2018). It is used to relieve abdominal pain, diarrhea, otitis media, and infections of the respiratory tract in Africa, notably Nigeria (Jaber and Al-Mossawi, 2007).

Carrots (Daucus carota L., family Apiaceae) are commonly cultivated as well as commercially exploited all over the world (Bawari et al., 2018). Carrots were ranked in the 10th place in nutritional value out of 39 vegetables and fruits (Dias, 2012). They are high in dietary fibre and the trace mineral molybdenum, which is uncommon in many vegetables. Carrots are also high in phenolics, polyacetylenes, and carotenoids and have a high nutritional value (Sharma et al., 2012). Carrots are also used medicinally to treat a variety of ailments. Carrot seed extracts have been shown to have hepato- and cardioprotective, antibacterial, antifungal, antiinflammatory, and analgesic properties (Jaber and Al-Mossawi, 2007)

Keywords: Proximate composition, Functional properties, Tomato, Garlic, Carrot.
Some experiments have reported the proximate and functional properties of the individual plants; tomatoes (Abdullahi et al., 2016), garlic (Ali and Ibrahim, 2019) and carrot, but no nutritional study on their combined formulations has been conducted. Therefore, due to the vast medicinal potential of these vegetables, this study is a preliminary one aimed at studying the proximate composition of tomato (S. lycopersicum), garlic (A. sativum) and carrot (D. carota) formulations in their individual forms and in mixture before they are experimentally evaluated as an antidiabetic in alloxan-induced diabetic wistar rats. In terms of mixing formulation of vegetables, it has been confirmed that, in certain situations, the organoleptic qualities of the formulation may be strengthened, in addition to the improved nutritional quality of the mixture as a result of the combination of two or more vegetables (Aderinola and Abaire, 2019).

MATERIALS AND METHODS

Collection of Samples
Fresh tomato, carrot and garlic samples were bought from Ajiwa Local Market (Latitude 12.9637° N and Longitude 7.7400° E) -- a market that operates weekly in Ajiwa village, Batagarawa Local Government Area of Katsina State, Nigeria. The samples were identified and authenticated at the Department of Plant Science, Bayero University Kano, Nigeria. Voucher specimens were kept in the department for future reference.

Sample preparation
To exclude the damaged ones, the tomato, carrot and garlic samples were carefully sorted out. Five hundred gram (500g) of each sample was picked and weighed using an electronic weighing balance (Weightech India MS and SS) in order to achieve homogeneity with respect to the weight of each sample. The samples were shed-dried and crushed to a fine powdered state using a local grinder (pestle and mortar). Samples that have been finely powdered were placed separately in a numbered sealed jar and preserved under standard laboratory conditions until required for reconstitution.

Analysis of the Proximate Composition
All the formulations were subjected to proximate analysis in accordance with the following established standard laboratory procedures (AOAC, 1990; AOCS, 2000). The proximate contents analyzed were moisture, crude protein, total ash, crude fiber, fat, and carbohydrate.

Analysis of the Functional Properties:
Glucose adsorption capacity (GAC) estimations
With minor modifications, the glucose adsorption capacity of the different formulations (samples) was evaluated using the method of Ou et al. (2001) and Chau et al. (2004). The concept was based on the fiber’s capacity to effectively attach to glucose, thereby reducing the amount of glucose in the system. In a nutshell, 1.0 g of each proportion (sample) was mixed individually in test tubes numbered 1-7 with 20 mL of prepared glucose solution (100 mg/mL). The mixtures were held for 90 minutes in a water bath at 37 °C, and it was centrifuged for 15 minutes at 3500 rpm. The amount of glucose in each supernatant was determined using the glucose/peroxidase technique at the end of the adsorption, and the amount of glucose adsorbed on the formulations (%) was computed as follows:
Amount of glucose that has been adsorbed (%) = (IG – FG / IG) × 100
Where: IG represents the initial concentration of glucose before adsorption, and FG is the final concentration of glucose after adsorption.
Oil adsorption capacity (OAC) and water adsorption capacity (WAC) estimation
The centrifugal method, as described by Chau et al. (2004), was used to measure the percentage water or oil adsorption capacities of the various formulations. In a nutshell, 1 gram of each formulation was weighed and placed into test tubes containing 20 mL distilled water or oil, and then thoroughly mixed before centrifuging for 15 minutes at 3500 rpm. At the end of the adsorption, the respective supernatant was measured, and the quantity of oil or water that was adsorbed by each formulation was determined as follows:
Quantity of oil or water adsorbed by each formulation (%) = (IA – FA / IA) × 100
Where: IA amount of oil or water initially before adsorption, and FA is the final amount of oil or water after adsorption.

Data Analysis
Results obtained were expressed as mean ± standard error of mean (SEM) and presented in tables. Statistical differences between groups were statistically analyzed by one-way analysis of variance (ANOVA), and then followed by Tukey’s post hoc test with the aid of SPSS version 25. P value of less than 0.05 (p < 0.05) was statistically considered to be significant.

RESULTS

Proximate Composition of Different Formulations (%) of Solanum lycopersicum (Tomato), Allium sativum (Garlic), and Daucus carota (Carrot).
Table 1. shows the proximate composition of different formulations of S. lycopersicum, A. sativum and D. carota. The result shows that tomato formulation had the highest value of moisture content (83.4 %), followed by carrot formulation with 82.0 % moisture content, and all the values were discovered to be significantly (p< 0.05) greater than the values of other formulations. The ash content was however observed to be significantly (p< 0.05) greater in garlic, T:G, G:C and T:G:C formulations when compared to the ash content of other formulations. Similarly, T:G formulation had the highest protein content (7.9 %) and it remained significantly (p< 0.05) higher in all formulations containing garlic when compared to the non-garlic containing formulations.

Table 1. Proximate composition of different formulations of tomato (S. lycopersicum), garlic (A. sativum) and carrot (D. carota) 

<table>
<thead>
<tr>
<th>Formulation/Parameter</th>
<th>Moisture</th>
<th>Ash</th>
<th>Crude protein</th>
<th>Fat</th>
<th>Crude fibre</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>83.4 ± 4.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9 ± 1.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.2 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4 ± 0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.9 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Garlic</td>
<td>65.0 ± 2.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.5 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Carrot</td>
<td>82.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.8 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.7 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.6 ± 0.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>T:G</td>
<td>65.5 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.9 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.3 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.3 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T:C</td>
<td>68.2 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.9 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>G:C</td>
<td>63.5 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6 ± 0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.9 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>T:G:C</td>
<td>61.4 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.4 ± 1.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.2 ± 1.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.6 ± 0.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.2 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

KEY: T:G (Tomato:Garlic); T:C (Tomato: Carrot); (Garlic: Carrot); T:C:G (Tomato:Carrot:Garlic); CHO (Carbohydrates). Values were expressed as mean ± standard error of mean (SEM). Mean values with different superscript letters within the same column are significantly (p < 0.05) different.

There was an observed significant (p< 0.05) decrease in fat content of the garlic formulation (0.5 %) when compared to that of all other formulations. Furthermore, crude fiber was

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significantly ($p < 0.05$) higher in all the mixture formulations (T:G, T:C, G:C and T:G:C) as compared to all individual formulations. On the other hand, carbohydrate was much higher in the garlic formulation (22.6 %) and T:G:C formulation (15.2%) and the values were observed to be significantly ($p < 0.05$) greater than that of other formulations. Tomato formulation had the least carbohydrate content (9.9%) as compared to other formulations.

### Functional Analysis of Various Formulations (% of Solanum lycopersicum (Tomato), Allium sativum (Garlic), and Daucus carota (Carrot)).

Table 2. shows the result of the functional analysis of different formulations of *S. lycopersicum, A. sativum* and *D. carota*. The glucose adsorption capacity of the different formulations ranged between 24.7 to 19.1. Tomato:Glucose (T:G) and T:G:C formulations had the highest glucose adsorption capacity that were observed to be significantly ($p < 0.05$) higher than that of the remaining formulations. On the contrary, no significant ($p > 0.05$) difference was observed among oil adsorption capacities of all the analyzed formulations. In terms of the water adsorption capacity, T:G formulation accounted for the highest water adsorption capacity (52.1 %) followed by tomato formulation (50.9%), even though, they were only observed to be significantly ($p < 0.05$) higher than garlic, carrot and T:C formulations.

#### Table 2. Functional properties of different formulations of Solanum lycopersicum, Allium sativum and Daucus carota

<table>
<thead>
<tr>
<th>Formulation/Parameter</th>
<th>GAC (%)</th>
<th>OAC (%)</th>
<th>WAC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>21.8 ± 0.4a</td>
<td>28.9 ± 0.1a</td>
<td>50.9 ± 0.3a</td>
</tr>
<tr>
<td>Garlic</td>
<td>20.4 ± 0.1a</td>
<td>27.3 ± 0.3a</td>
<td>47.5 ± 1.0b</td>
</tr>
<tr>
<td>Carrot</td>
<td>19.1 ± 0.2a</td>
<td>26.4 ± 0.3a</td>
<td>46.4 ± 0.2b</td>
</tr>
<tr>
<td>T:G</td>
<td>24.7 ± 0.4b</td>
<td>29.3 ± 0.1a</td>
<td>52.1 ± 0.5a</td>
</tr>
<tr>
<td>T:C</td>
<td>21.4 ± 0.1a</td>
<td>28.2 ± 0.1a</td>
<td>47.9 ± 0.4b</td>
</tr>
<tr>
<td>G:C</td>
<td>20.2 ± 0.1a</td>
<td>27.3 ± 0.1a</td>
<td>49.3 ± 0.2b</td>
</tr>
<tr>
<td>T:G:C</td>
<td>23.5 ± 0.0b</td>
<td>26.8 ± 0.4a</td>
<td>48.6 ± 0.4b</td>
</tr>
</tbody>
</table>

**KEY:** T:G (Tomato:Garlic); T:C (Tomato: Carrot); (Garlic: Carrot); T:G:C (Tomato:Carrot:Garlic); GAC (glucose adsorption capacity); OAC (oil adsorption capacity); WAC (water adsorption capacity). Values were expressed as mean ± standard error of mean (SEM). Mean values with different superscript letters within the same column are significantly ($p < 0.05$) different.

### DISCUSSION

The growing popularity of plant-based formulations has resulted in a rapidly expanding market for ayurvedic, nutraceutical, and polyherbal formulations; however, due to the large number of chemical compounds found in the various medicinal plants, majority of such items have their quality uncontrollably left (Pari and Murugan, 2005). Plants play an important function in the treatment of diabetes mellitus and other degenerative disorders. Tomato, garlic and carrot plants are known to provide a variety of benefits to human health, including the treatment of constipation and other inflammatory illnesses. Also, they have been shown to possess a potent antidiabetic impact (Eidi et al., 2006; Debjit et al., 2012).

In the proximate composition analysis, food deterioration is primarily determined by moisture content. It is generally known that, reduced moisture content in food samples lowers microbial activity and hence extends the shelf life of food products (Ismaila et al., 2018). The high moisture and low ash contents in tomato reported in this study is supported by the work of Abdullahi et al. (2016) who reported high moisture (93.8 %) and low ash (0.85 %) contents in tomato in their study of the proximate, mineral and vitamin composition of fresh and canned tomato. The T:G:C formulation had the lowest moisture content, implying that it may
be stored for a long time. Water is an important component of almost every diet, serving not only as a filling material and solvent, but also as an essential means of maintaining macromolecule and cell activities and structure (Kaatze and Hubner, 2010). As a result, understanding moisture content is critical for predicting food behavior throughout processing, storage and consumption. Ash content is an indication of the total inorganic components after the elimination by oxidation or combustion of moisture and organic materials (fats, proteins and carbohydrates) in a muffle furnace (Iwe et al., 2016). When assessing levels of essential minerals, the content of ash present in a dietary sample plays an essential role (Thomas et al., 2013). The highest crude protein content in T:G and G:C formulations suggested that the aim of fortification which was to improve the content of the protein in some of the mixed formulations was achieved while concurrently producing stable formulations due to their low moisture content. On the other hand, garlic and T:G:C formulations had the lowest fat content among all other formulations. Fats content influences product stability if it exceeds the desired level since unsaturated fatty acids are more susceptible to oxidative rancidity than saturated fatty acids (Tenagashaw et al., 2015). Low crude fibre content recorded in single formulations in this study is in agreement with the work of Ali and Ibrahim (2019) who also reported low crude fibre content in single garlic formulation (3.96 %). The significant (p < 0.05) high increase in crude fiber content recorded in the mixed formulations is beneficial because fiber has been shown to have physiological effects on the gastrointestinal system such as the removal of neutral steroids and bile acids, all of which lower the body cholesterol pool (Etiosa et al., 2017).

In the functional properties evaluation of the formulations, Glucose Adsorption Capacity (GAC) of the formulations was studied because it serves as a measure of a fiber’s ability to absorb glucose and thereby lower the glucose levels in the surrounding environment. This may be due to the architecture of the fiber rather than its chemical content (Ismaila et al., 2018). The highest value of the GAC recorded in T:G and T:G:C formulations could be ascribed to the increase in water holding capacity and viscosity of the fibers. This also indicated that fiber could aid glucose retention in an in vitro setting. Similar effect might be achieved in vivo, which could be useful in terms of lowering the quantity of glucose absorbed from the small intestine. Ismaila et al. (2018) findings on okra fruits and Ramadan et al. (2017) findings on cucumber and ginger fruits are similar to the present result. Fiber matter, according to Lopez et al. (1996), may promote glucose entrapment inside the fiber matrix by reducing water mobility on the surface and, as a result, the fiber particles’ hydration ability is improved, possibly contributing to glucose retention on the fiber surface.

Water adsorption capacity (WAC) is a useful parameter that serves as index for predicting a fiber materials’ fecal bulking ability, where as OAC is an index for predicting fiber matter’s lipophilic behavior. Water adsorption capacity is solely dependent on the availability of hydrophilic groups that attract water molecules, as well as the macromolecules’ ability to form gels (Twinomuhwezi et al., 2020). Water adsorption capacity is significant in foods because it affects emulsification, adhesion, solubility, dispersibility, wettability, cohesion, viscosity, and gelation, among other functions (Obiegbuna et al., 2019).

**CONCLUSION**

The present study has established the proximate composition and functional properties of *S. lycopersicum*, *A. sativum* and *D. carota* formulations. There was an observed significant (p<0.05) difference in the proximate composition and functional properties of the various formulations. The moisture content was highest in tomato formulation (83.4 %) and lowest in T:G:C formulation (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %). Ash content was observed to be higher in all garlic containing formulations (61.4 %).
formulations while crude protein content was observed to be greater in T:G formulation (7.9 %) and least in tomato formulation (1.8 %). Fat content was observed to be nonsignificant ($p > 0.05$) among all the formulations with the exception garlic formulation. Crude fiber was significantly higher in all the mixed formulations than the single formulations. Highest carbohydrate content was recorded in garlic formulation while the least content of it was recorded in tomato formulation. The functional analysis revealed a significant variation in GAC, OAC and WAC among all the formulations that were tested. The outcomes of this study have led us to the conclusion that employing safe combinations of tomato, garlic, and carrot formulations for the production of nutraceuticals is recommended

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Conflict of Interest
The authors declare no conflicts of interest

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