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Antidiabetic and Antioxidant Properties of Methanolic Extract for a Diabetic Supplement Developed from Drumsticks Leaves (Moringa Oleifera), Garlic (Allium Sativum) and Cinnamon (Cinnamomum Verum) Powders

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Abstract
Diabetes Mellitus is a progressive and chronic metabolic disorder which is defined by high blood sugar levels. The disease is a result of insufficient insulin hormone secretion or its action. One of the methods to control blood glucose level is to inhibit the action of alpha amylase and alpha glucosidase enzymes in order to slow down or decrease hydrolyze of carbohydrates and therefore management of hyperglycemia in diabetes type II patients. This will help to avoid long and short term consequences of the disease. Plants containing great amounts of phenolic compounds are natural, accessible and cheap sources of α-glucosidase and α-amylase inhibitors. At the same time, the present food supplements made up of natural ingredients has potential to possess antioxidant activity as well. The present research evaluated the antidiabetic effects and antioxidant activity of *the* methanolic extract of the *supplement* powders developed from three food ingredients including Drumsticks Leaves (*Moringa Oleifera*), Garlic (*Allium Sativum*) and Cinnamon (*Cinnamomum Verum*) with different percentages to determine the antidiabetic and antioxidant properties of the supplement to manage blood glucose levels of type II diabetes. The inhibition activity of alpha amylase enzyme is ranged between 97 to 99% for the food ingredients and 95 to 99% for the supplements. The value of inhibition percentage for drumstick leaves is 98.36% ± 0.09. Values observed for cinnamon and garlic were 99.2% ± 0.27 and 97.52% ± 2.05 respectively. The inhibition activity of the supplement no. 3 containing 70% dried drumstick leaves powder + 15% cinnamon powder and 15% garlic powder shows the highest inhibiting activity of 99.2%. The α-glucosidase enzyme inhibition activity for drumstick leaves is 33.64%, cinnamon 26.73% and garlic 22.64% while the supplements activity are ranged between 15.55 to 28.69%. Supplement no. 3 has the highest inhibition activity of 28.69%. DPPH radical scavenging activity of methanolic extracts was performed in 3 different concentrations of 10, 20 and 30µg of each sample extract. In this research, the activity of drumstick leaves in 10µg/ml show 74.65%, 79.67% in 20µg/ml and 76.17% in 30µg/ml. The obtained values for cinnamon are 85.98% in 10µg/ml, 90.33% in 20µg/ml and 89.81% in 30µg/ml while the radical scavenging activity of garlic is 72.57% in 10µg/ml, 67.92% in 20µg/ml and 63.84% in 30µg/ml. DPPH radical scavenging activity of the supplements are ranged from 66.96 to 71.90% in 10µg/ml, 65.62 to 80.04% in 20µg/ml and 73.16 to 80.96% in 30µg/ml as shown in Table 4. Supplement no. 2, containing 80% dried drumstick leaves powder, 10% cinnamon powder and 10% garlic powder in 30µg/ml concentration shows the highest radical scavenging activity, 81%, in comparison with other supplements in different concentrations. The research proved that the formulated supplements have high antidiabetic and antioxidant properties. Therefore, the products can be consumed by type II diabetic patients as a safe, low calorie, natural and low-price supplement to manage blood glucose levels and prevent the long and short time consequences of the unmanaged disease and accordingly retard and decrease complications during lifetime.
Keywords: Antidiabetic activity, Antioxidant property, Methanolic extraction, Moringa Oleifera, Allium Sativum, Cinnamomum Verum, Supplements.

1. Introduction

The epidemic of diabetes type II, as a public health global crisis threatens population's economies and health specially developing nations. The crisis accelerates by more sedentary lifestyle, rapidly urbanization and nutrition habits changes. Obesity and overweight increment helps the current situation gets even worse globally (HU, 2011).

Diabetes is continuously increasing in the world not only in rich populations but also in countries with middle income (Chan, 2016). DM as a serious public health problems is one of the four noncommunicable (NCDs) diseases that the leaders in the world have targeted. During the past decades, the incidence and prevalence of the disease have been on a rise constantly. It is estimated that the number of adult diabetes cases has been elevated from 108 million in 1980 to 422 million patients in 2014 with the high prevalence of 8.5% which is predicted to reach over 592 million in 2035. In 2012, a total number of 3.7 million deaths was reported caused by diabetes. The prevalence rate in middle and low income countries has shown a faster increment than the high income populations during the last 30 years (World Health organization, 2016). Glycemic management

can decrease the rate of complications of the disease like nephropathy, neuropathy, retinopathy, cardiovascular problems, limb amputation and death. Each 1% decrement of HbA1C, can effectively lower about 35% in microvascular problems and 25% death rate related to diabetes type II (American Diabetes Association, 2000). Metformin is the most known and first line medicine to treat diabetes which is accompanied with side effects such as gastrointestinal discomfort, vomiting, nausea and diarrhea (Kim *et al*., 2012; McCreight, Bailey and Pearson, 2016; Bonnet and Scheen, 2017). Meglitinides, gliclazide and glimepiride as sulfonylureas, can cause hypoglycemia and weight gain (Tran *et al*., 2015). Medicine's Agency (EMA) has suppressed the authorization for marketing Thiazolidinediones (e.g. pioglitazone) because of its cardiovascular risks (National Institute for Health and Clinical Excellence, 2009). Tran and others (2015) reported the increased risk of bladder cancer and heart failure with Thiazolidinediones intake. Well-timed commencement and suitable intensification of insulin injection is a challenge in DM management (Bajaj, 2018). Insulin qua a blood glucose level in DM treatment, herewith the fear and pain on frequent injections, difficulty of the regimens and worries about the self-management failure are considered as the obstacles in insulin therapy initiation (Fonseca and Haggar, 2014).

There are many ways of treating and managing type II diabetes. Nowadays, there is more attention on natural options rather than the chemical and synthetic drugs to both treating and managing the disease. Combined herbal supplementation is one of the best methods which can be helpful to control blood glucose levels, possessing great health benefits while being cost effective for both diabetics and healthy people. There are various types of natural supplements which can be used to manage diabetes type II. Drumstick leave is a rich source of nutraceuticals with high biological capacity to effect on chronic diseases such as diabetes, cancer, inflammation, microbial infections, etc. (Udechukwu, 2018). Cinnamon verum is a potential healing factor with several useful effects in managing diabetes type II (Ranasinghe *et al*., 2012). Garlic, a traditional herbal remedy with various health benefits can effectively manage glucose levels and act as an antioxidant (Tsai *et al*., 2012).

This study sought to analyse the potential of drumstick leaves, garlic and cinnamon utilization in developing a low calorie supplement with high antidiabetic and antioxidant activity for type II diabetes management.

2. Materials and methods

2.1 Chemicals

Petroleum ether (Fisher scientific UK, Code: P/1760/17, Lot: 121048), Methanol extra pure (Loba Chemie, batch no.: LB020707), Hydrochloric Acid 35.4% (Lot no.: A164061506), Formic acid 98% (Lot no.: A154251501).

2.2 Ingredients

The three food ingredients used to formulate the present food supplements were chosen for their known anti-diabetic properties including drumsticks leaves (*Moringa oleifera*), garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*) (Table 1).

2.3 Sample collection and preparation

Samples were collected including drumsticks leaves (*Moringa oleifera*), garlic (*Allium sativum*) and cinnamon (*Cinnamomum verum*). The fresh drumstick leaves were collected from Chiromo campus, University of Nairobi, Nairobi, Kenya. The cinnamon barks and fresh garlic were purchased from Open air markets, Westlands, Nairobi, Kenya.

The drumstick leaves were collected directly from the tree, washed and rinsed with distilled water, dried in air Oven (Memmert GmbH+Co.KG, D-91126 Schwabach FRG, Germany. type: UE 500, F.-Nr.: c504.0495) at 60-65˚C for 3:30 hours. Then milled by home miller (VON Hotpoint, model no.: HB251KW, China) and sieved through 100 micron sieve to obtain a fine powder. The fresh Garlic was cleaned and size reduced, dried at 60-65˚C for 6 hours in air oven. Milled and sieved through 100 micron sieve. The cinnamon barks washed, dried in air oven at 60-65˚C for 4 hours, milled and sieved through 125 micron sieve. The process flow diagram for product formulation is shown in Figure 1.

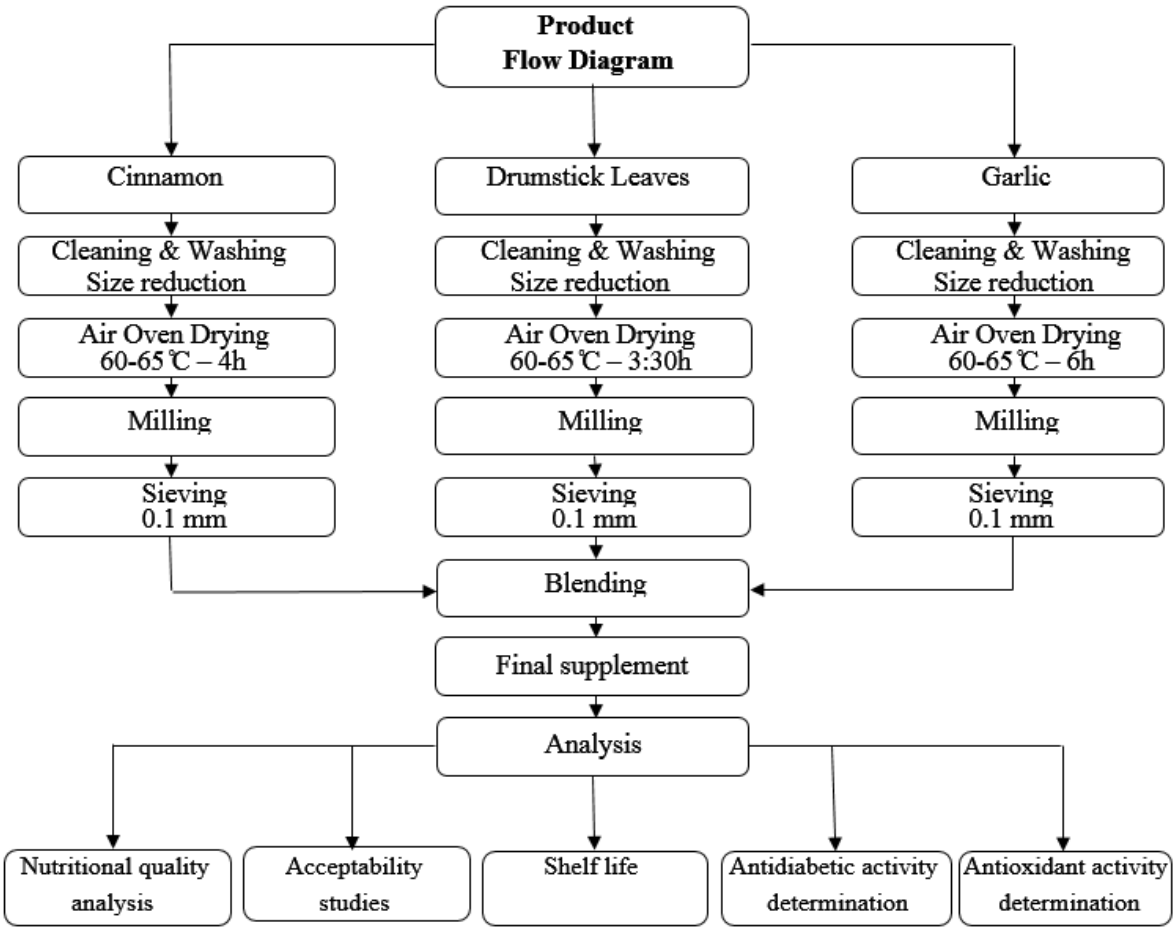

Figure 1. Process flow diagram for the product formulation

2.4 Methanolic extract preparation

1.25g of each sample was defatted by adding 125ml petroleum ether, in 1:10 (w/v) ratio. The samples were kept in normal water bath (Memmert, type: WB 22, F.Nr.: 1505.0320, Memmert GmbH+Co.KG, Germany) at 50-60° C for 30 minutes. The samples were centrifuged (Model Supra 22K, serial no.: 4241227, Human lab instruments co., Korea) at 13,000rpm for 5 minutes. All the supernatants were discarded and the residue was air dried overnight. 1g of each defatted sample was extracted by sequentially adding 10ml of HCL-methanol (1ml/100ml) and then by adding 10ml of HCL-methanol-water (1ml/79ml/100ml) and 10ml of HCL-methanol-water (1ml/49ml/100ml) in the water bath at 50-60° C for 30 min. The samples were centrifuged at 13,000rpm for 5 min. The supernatants were pooled together after each extraction. Each sample extract was eluted by adding 10ml of methanol-water. Methanol was removed by a rotary vacuum evaporator at 337mbar pressure at 40° C for 30-35 min each sample. Then samples were freeze dried by lyophilizer for 24 hours at -10° C, +35mbar. The residue was weighed and the total dry yield of the extract calculated. Each sample extract was re-dissolved in water:methanol:formic acid (47.5:47.5:5, v/v/v) solution the ratio of 1mg of extract per mililiter of solvent and used for further tests.

2.5 Supplement formulation

Linear programming model was used to blend the different proportions of the food ingredients and in selection of an optimized supplement powder in quantity of nutrients including vitamins and minerals according to RDA. The four selected formulations selected in different proportions including S1: Dried Moringa leaves powder 90% + Cinnamon Powder 5% + Garlic Powder 5%. S2: Dried Moringa leaves powder 80% + Cinnamon Powder 10% + Garlic Powder 10%. S3: Dried Moringa leaves powder 70% + Cinnamon Powder 15% + Garlic Powder 15%. S4: Dried Moringa leaves powder 50% + Cinnamon Powder 25% + Garlic Powder 25%). The samples were prepared and labeled as shown in Table 1.

Table 1. Food ingredients and supplements percentages

Supplement no.	Food ingredients (%)
I1*	Dried Drumstick leaves powder - 100%
I2	Cinnamon powder - 100%
I3	Garlic Powder - 100%
S1**	Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%
S2	Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%
S3	Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%
S4	Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%

*I= Ingredient **S = Supplement

2.6 Antidiabetic effect

2.6.1 α-Amylase inhibition activity assay

Worthington (1993) method was used to measure the α-Amylase inhibition activity of each ingredient and the supplements. The methanolic extract of each sample volume (500 µl) and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) (0.5 mg/ml) were incubated at 25°C for 10 min. The mixture of Sodium phosphate buffer 0.02M + Sodium chloride 0.006M (pH 6.9) and 500µl starch solution 1% (1g starch + 100ml distilled water) was prepared and regularly added up to all test tubes. Thereafter, incubation for 10 minutes at ambient temperature (25° C), the reaction came to a standstill after adding 1ml color reagent (dinitrosalicylic acid). Afterwards, the tubes were incubated for 5 minutes at 95 °C water bath and left to cool at room temperature. Adding distilled water (15ml), the reaction was diluted. Using the spectrophotometer (JENWAY 6305), absorbance recorded at 540 nm. Readings were compared with the control containing buffer in place of sample extract. The percentage of enzyme inhibitory activity of the aqueous extracts was calculated according to the following formula:

$$Inhibition\ (\%) = (A_{control} - A_{sample}) / A_{control} \times 100 \quad (1)$$

2.6.2 α-Glucosidase inhibition activity assay

100 µl Phosphate buffer 0.1M, pH 6.9 consisting α-glucosidase solution (1unit/ml) and 50 µl of each extract sample (100µg/ml) were transferred to the 96-well flat-bottom microtitration plate and then pre-incubated for 10 min at 25° C. Frequently was added to each well 50µl of 5 mM p-nitrophenyl-α-D-glucopyranoside (PGPP) solution in 0.1M phosphate buffer pH 6.9. The reaction mixture incubated for 5 min at 25° C. Afterwards, the absorbance readings were noted at 405nm using Eliza reader (ref. no.: 51118170) and were compared with the control containing 50 µl buffer solution instead of sample extract. The percentage of α-glucosidase inhibition activity was calculated using the formula below:

$$Inhibition\ (\%) = (A_{control} - A_{sample}) / A_{control} \times 100 \quad (2)$$

2.7 Antioxidant activity

2.7.1 DPPH radical scavenging activity

The antioxidant activity of each methanolic extract was evaluated according to Sanchez-Moreno *et al.* method (1998) with some modifications. 500 µl of DPPH methanolic solution (0.0157g/200 ml methanol) was added to 3 different concentrations of 10, 20 and 30 µg/ml of each sample extract. After incubation at 25° C for 30 mins, the readings were measured at 515nm with spectrophotometer. The tested sample's radical scavenging activity was calculated as a decline in the absorbance of DPPH radical scavenging activity:

$$DPPH\ radical\ scavenging\ activity\ (\%) = (1\text{-}A\ sample / A\ negative\ control) \times 100 \quad (3)$$

2.8 Statistical analysis

Each test was repeated in triplicate (n=3). The results presented as mean ± SD. Data analysis was done using two-way ANOVA to specify the significant differences between each supplement using the pure ingredient as the control. SPSS (version 23) was used to perform statistical analysis.

3. Results and discussion

3.1 Antidiabetic activity

3.1.1 α-Amylase inhibition activity assay

In the present research, α-amylase enzyme inhibition activity of the methanolic extracts of Drumsticks Leaves (*Moringa oleifera*), Garlic (*Allium sativum*) and Cinnamon (*Cinnamomum verum*) and the formulated supplements were studied.

The enzyme inhibition activity percentage of the methanolic extracts tested in this study, is shown in Table 2. The inhibition percentage was ranged between 97 to 99% for the three food ingredients and 95 to 99% for the

supplements. The value of inhibition percentage for drumstick leaves was 98.36% ± 0.09, cinnamon 99.2% ± 0.27 and garlic 97.52% ± 2.05. The inhibition activity of supplement no. 3, containing 70% dried drumstick leaves powder + 15% cinnamon powder and 15% garlic powder showed the highest value of 99.2% ± 0.72. There was overall significant difference between the ingredients and supplements (p-value < 0.05). Ingredients no. 1, 3 and supplement no. 4 are not significantly different in (p-value > 0.05).

Table 2. α-Amylase enzyme inhibition activity of the methanolic extract of food ingredients and formulated supplements

Food ingredients and supplements	**α-amylase inhibition activity (%)***
I1- Dried Drumstick leaves powder - 100%	$98.36^{a} \pm 0.092$
I2- Cinnamon powder - 100%	$99.20^{b} \pm 0.271$
I3- Garlic Powder - 100%	$97.52^{c} \pm 2.052$
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	$98.73^{d} \pm 0.890$
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	$95.21^{abcdef} \pm 1.589$
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	$99.20^{e} \pm 0.722$
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	$97.95^{f} \pm 0.890$

*The values in the table are means of the triplicates ± SD

- The values whose superscript are different letters, are significantly different at (p-value < 0.05)

The recent researches have proved the high ability of α-amylase inhibition activity as a natural alternative for synthetic drugs in type II diabetes management. Drumstick leaves have shown remarkable α-amylase inhibitory activity due to its high phenolic contents (Jimoh, 2018). Verspohl and others (2005) and Sudha and others (2011) have also described α-amylase inhibition activity of both cinnamon and garlic as a blood glucose reducers and insulin level increasers.

There are number of therapeutic ways of diabetes type II management such as insulin demand reduction, insulin secretion stimulation, insulin action improvement at tissue level and controlling the action of breakage of disaccharides and oligosaccharides (Funke & Melzing, 2006). One of the effectual ways of diabetes management is to control hyperglycemia through decelerating the digestion of consumed carbohydrates by inhibiting the CHO degrading enzymes. This can notably decrease postprandial blood sugar level after each food intake (Tundis *et al*., 2011).

α-amylase enzyme acts as a catalyzer at the first stage in starch hydrolysis to maltose. At the next step maltose breaks to glucose by α-glucosidase enzyme's action as the catalyzer. Accordingly, retardant action of α-amylase in starch digestion, can effectively control hyperglycemia after each meal in patients with type II diabetes (Brayer *et al*., 1995; Tundis *et al*., 2011).

3.1.2 α-Glucosidase inhibition activity of food ingredients and supplements

α-glucosidase enzyme inhibition activity of methanolic extracts in this research, was between 22 to 33% for the three ingredients. Drumstick leaves showed 33.64%, cinnamon 26.73% and garlic 22.64% inhibition activity and the supplements were ranged from 15.55 to 28.69%, shown in Table 3. Supplement no. 3 demonstrates the highest activity with 28.69% inhibition amongst the other supplements. There was no overall significant difference between the ingredients and supplements (p-value > 0.05). Supplements no. 1, 2 and 3 are not significantly different in (p-value > 0.05).

Table 3. α-Glucosidase enzyme inhibition activity of the methanolic extract of food ingredients and supplements

Food ingredients and supplement	**α-glucosidase inhibition activity (%)***
I1- Dried Drumstick leaves powder - 100%	$33.64^{a} \pm 0.551$
I2- Cinnamon powder - 100%	$26.73^{b} \pm 0.622$
I3- Garlic Powder - 100%	22.64 ± 1.333
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	$28.34^{c} \pm 1.945$
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	25.86 ± 3.108
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	28.69 ± 11.370
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	$15.55^{abcd} \pm 8.179$

* The values in the table are means of the triplicates ± SD

-The values whose superscript are different letters, are significantly different at (p-value < 0.05)

Previous researches have shown that one of the effective curative ways in controlling blood glucose level in DM is to manage postprandial hyperglycemia by alpha glucosidase enzyme inhibition. This enzyme catalyzes the action of carbohydrates hydrolyzing; therefore its inhibition will end up to retard glucose absorption and

avoids hyperglycemia. Many research works have been done through recent years to find effective natural inhibitors of alpha glucosidase (AGI) to substitute the synthetic medicines in order to avoid the side effects like diarrhea, stomach pain, flatulence and bloating. Plants secondary metabolites are rich phytochemical sources like phenolic compounds, anthocyanins, flavonoids, terpenoids, glycosides and alkaloids having appreciable inhibitory activity against α-glucosidase enzyme (Bukhari *et al*., 2017).

Dried drumstick leaves powder can effectively inhibit the action of α-glucosidase enzyme and therefore has the potential to act as a natural food source to manage type II diabetes. Although the composition of its chemical compounds degrades after the drying process (Natsir *et al.*, 2018). Cinnamon contains phenolic phytochemicals which inhibits the action of α-glucosidase, the carbohydrate hydrolyzing enzyme (Brown *et al.*, 2017). Garlic possess antidiabetic properties through its inhibitory effect on α-amylase and α-glucosidase accompanied by the ability of lipid peroxidation prevention in heart and pancreas which explains its high antioxidant ability (Oboh *et al.*, 2018). The aim of the present research was to develop a food supplement with the potential of AGI which can work as a functional food containing phenolic compounds to well manage diabetes with a low price and locally available ingredients.

3.2 Antioxidant activity

The DPPH radical scavenging activity of methanolic extracts was performed in 3 different concentrations of 10, 20 and 30µg/ml of each sample extract. In this research, the activity of drumstick leaves in 10µg/ml showed 74.65%, 79.67% in 20µg/ml and 76.17% in 30µg/ml. The inhibition values for cinnamon were 85.98% in 10µg/ml, 90.33% in 20µg/ml and 89.81% in 30µg/ml while the radical scavenging activity of garlic was 72.57% in 10µg/ml, 67.92% in 20µg/ml and 63.84% in 30µg/ml. DPPH radical scavenging activity of the supplements were ranged from 66.96 to 71.90% in 10µg/ml, 65.62 to 80.04% in 20µg/ml and 73.16 to 80.96% in 30µg/ml as shown in Table 4. Supplement 2, with 80% dried drumstick leaves powder, 10% cinnamon powder and 10% garlic powder in 30µg/ml concentration showed the highest radical scavenging activity 81%, comparing the other supplements in different concentrations. There was significant difference (p-value < 0.05) between 10 and 30µg/ml in Supplement no. 2.

Table 4. Antioxidant activity of the methanolic extract of food ingredients and formulated supplements

Food ingredients and supplement samples	**DPPH radical scavenging activity (%)***		
	10µg/ml	**20µg/ml**	**30µg/ml**
I1- Dried Drumstick leaves powder - 100%	74.65 ± 3.54	79.67 ± 5.93	76.17 ± 2.06
I2- Cinnamon powder - 100%	85.98 ± 1.12	90.33 ± 2.53	89.81 ± 5.01
I3- Garlic Powder - 100%	72.57 ± 3.03	67.92 ± 6.74	63.84 ± 11.26
S1- Dried Drumstick leaves powder 90% - Cinnamon Powder 5% - Garlic powder 5%	70.01 ± 2.55	65.62 ± 10.43	74.24 ± 3.17
S2- Dried Drumstick leaves powder 80% - Cinnamon Powder 10% - Garlic powder 10%	71.90 ± 4.77	76.69 ± 3.98	80.96 ± 1.31
S3- Dried Drumstick leaves powder 70% - Cinnamon Powder 15% - Garlic powder 15%	66.96 ± 9.52	80.04 ± 15.50	73.16 ± 1.13
S4- Dried Drumstick leaves powder 50% - Cinnamon Powder 25% - Garlic powder 25%	71.64 ± 7.76	73.13 ± 10.14	73.16 ± 10.60

* The values in the table are means of the triplicates ± SD

Drumstick leave is a rich source of phenolic antioxidant compounds like quercetin, gallic acid, kaempferol and flavonoids which enables the human body to improve antioxidant system of free radical scavenging ability (Santos *et al*., 2012; Fitriana *et al*., 2016). Many researches have approved antidiabetic and antioxidant activity of cinnamon along with its antimicrobial, anti-inflammatory, antifungal, mosquito larvicidal, antimycotic, anticancer, nematicidal, antitermitic and insecticide properties (Benzie & Strain, 1999; Bruni *et al*., 2004; Cherioconi *et al*., 2005; Butt *et al*. 2009; Shahid *et al*., 2018). Garlic is known for its various health benefits and antioxidant properties (Galano and Francisco-Marquez, 2009). It is proved that garlic extracts are potential natural antioxidants (El-Hamidi & El-Shami, 2015).

DPPH radical scavenging activity is a very common method to evaluate the antioxidant activity of all liquid or solid food samples to measure the capability of the free radical scavengers of food compounds or hydrogen donors. DPPH is used to assess the overall antioxidant capacity of a food sample to specify a special antioxidant component (Parasad *et al*., 1995). The modified method of Sanchez-Moreno *et al*. (1998) was used in this study.

4. Conclusion

The research proved that the food ingredients and formulated supplements have high antidiabetic and antioxidant properties. Therefore, the products can be consumed by type II diabetic patients as a safe, low calorie, natural and low-price supplement to manage blood glucose level and prevent the long and short time consequences of the unmanaged disease and as a result retard and lower its complications during patient's lifetime.

Conflicts of interest: The author of this article has no conflicts of interest.

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