

Phytochemical Composition and Anti-Prostate Cancer Potentials of Two Different Ginger (*Zingiber Officinale*) Samples Grown in Parts of Oru-East LGA, Imo State, Nigeria

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Abstract

This study was designed to determine the phytochemical composition and anti-prostate cancer potentials of some ginger samples grown in parts of Oru-East LGA. Twenty different samples i.e (red and brown) ginger samples were used in the study. The samples were analyzed using standard biochemical methods. The results of the phytochemical analysis revealed the presence of alkaloids (11.60 ± 0.22 and 13.20 ± 0.2), saponin (9.20 ± 1.00 and 7.20 ± 1.66), tannin (13.20 ± 1.10 and 11.0 ± 0.41), flavonoid (8.8 ± 0.2 and 10.6 ± 0.22), reducing sugar (6.2 ± 0.22 and 9.30 ± 0.32) and polyphenol (21 ± 0.66 and 18.60 ± 0.11) respectively. There was no significant difference ($p > 0.05$) in the phytochemical composition of the two ginger samples. The study also revealed the presence of some mineral elements like zinc (4.19 ± 0.36 and 2.0 ± 0.11), manganese (28 ± 0.90 and 15.28 ± 0.22), copper (0.80 ± 0.1 and 0.94 ± 0.52), calcium (34.5 ± 1.99 and 45.0 ± 1.22), phosphorus (26.30 ± 1.59 and 28 ± 0.52), iron (6.5 ± 0.88 and 5.8 ± 0.55), sodium (38.26 ± 0.44 and 33.0 ± 2.06) and potassium (36.34 ± 1.94 and 22 ± 1.22) respectively. There was a significant difference ($p > 0.05$) in the level of zinc, manganese, iron, sodium and potassium in sample A when compared with those of red ginger. The result of the proximate analysis revealed the presence of very important bioactive compounds like 6-gingerol (15 ± 0.22 and 18.0 ± 0.13), 6-shogaol (12.0 ± 0.05 and 14.9 ± 0.11) and paradol (2.5 ± 0.20 and 1.5 ± 0.02) respectively. Ash (6.0 ± 1.20 and 4.4 ± 2.10), moisture (28 ± 0.70 and 22 ± 1.66), crude fibre (14 ± 2.11 and 12 ± 0.91), lipid (12.0 ± 0.80 and 6.0 ± 0.91), crude protein (2.5 ± 0.32 and 2.0 ± 0.10) and carbohydrate (38 ± 0.32 and 3.6 ± 0.11) respectively. The study also recorded a significant decrease in the level of survivin cyclin D1, cyclin B1, cyclin E and C-MYC proteins levels after treatment of the tumour induced albino rats with increasing concentrations of whole ginger extract. These findings suggest that whole ginger extract could be used in prevention and management of prostate cancer with tolerable side effects.

Keywords: Ginger, Prostate Cancer, Gingerol, Shogaol, Paradol.

Introduction

Prostate cancer is the second most common cancer among Nigerian men and the world at large (Canie, 2016). The prevalence of prostate cancer increases with age especially in men who are fifty (50) years of age and above. In addition, men with family history of the disease are at higher risk of developing prostate cancer (Muller, et al., 2020).

Nigerian men constitute about 15.2% of prostate cancer cases in sub-Saharan Africa. This indicates that despite the awareness and screening programmes by governments and

NGOs, the number of patients with prostate cancer have continued to increase (Angos et al., 2018).

Early prostate disorder has no symptoms, but can be detected through clinical examination and laboratory investigations. As the disease progresses, the patients may experience weak or interrupted urine flow, inability to urinate or difficulty in starting or stopping urine flow, blood in urine with severe pain.

However, these symptoms are not specific to prostate cancer alone, but may be due to benign cases such as prostate hyperplasia and prostatitis (Angos et al, 2018). Therefore, specific diagnosis depends on accurate histopathological examination to and quantitative prostate specific antigen assay. Thus sufficient evidence suggests that certain phytochemicals present in plant-based foods and dietary agents may work through complementary mechanism for cancer prevention and therapeutic benefits (Canie et al, 2016).

With this mindset, we undertook a detailed evaluation of *in vivo* anti-prostate cancer activity of locally grown ginger (*Zingiber officinale*) and the result of the study has provided adequate information on prevention and treatment of prostate cancer with natural therapeutics like ginger.

Materials and Methods

Animals: White albino rats were used in this study. The animals were obtained from the Animal Science Department of Michael Opara University of Agriculture, Umudike.

Preparation of Ginger

The ginger samples were sourced from local farmers in parts of Oru East L.G.A. They were identified by Dr. Onuoha C., a botanist in the Department of Botany and Biotechnology of Imo State University Owerri.

The ginger extracts were prepared by soaking ground ginger in methanol over night for three consecutive days. The supernatants were collected daily and finally concentrated followed by freeze drying using Lyophilizer.

Proximate Analysis

Proximate analyses were carried out in all the ginger samples. Moisture, ash, crude film, lipid, crude proteins, carbohydrate, gingerol, shogaol and paradol were determined by the method adopted by Angos et al (2018).

Mineral Elements Analysis

The mineral elements were determined first by ashing the ginger samples as describe by Osobo et al (1996). Sodium and potassium were determined using flame photometer (model – FP910).

Iron, copper, zinc, calcium, manganese and phosphorus were also determined by atomic absorption photometer (Model – L15310).

Phyto Chemical Screening

The phytochemicals screening for the presence of alkaloids, saponins, tannins flavonoids, reducing sugar and polyphenols were carried out according to the method adopted by Harbor et al (2018).

Invivo Tumour Growth and Treatment

Albino rats were used in the study. About 10ml of PBS with PC₃-cells were injected subcutaneously in the right flank region of the rats. Two weeks later, the rats developed visible tumours and were treated with 50mg, 100mg, 150mg, 200mg and 250 mg/ml of ginger extract daily by oral administration for one month.

Immunoblot Assay

Proteins were resolved using polyacrylamite gel electrophoresis and transferred into polyvinylidene difluoride membrane.

The membranes blocked in tris-buffer saline containing 0.5% tween -20 and 5% fat free dry milk, then with horse radish peroxidase conjugal secondary antibodies according to manufacturer's instructions (Birtalinly reagents xv. USA).

Statistical Analysis

The results were expressed as mean and standard deviation values using ANOVA and student's t-test, p-value of less than 0.05 were considered significant.

Results

Table I below shows the result of mineral compositions of the two ginger (*Zingiber officinale*) samples examined.

The results of the study revealed the presence of zinc 4.18 ± 0.6 , manganese 18.90 ± 1.6 , copper 0.80 ± 0.10 , calcium 34.55 ± 1.99 , phosphorus 26.30 ± 1.56 , iron 1.59 ± 0.58 , sodium 38.16 ± 3.57 , and potassium 26.34 ± 1.93 (m/l) in Sample A, as well as the presence of Zinc 2.20 ± 0.11 , manganese 15.08 ± 0.22 , copper 0.9 ± 0.52 , iron 2.8 ± 0.55 , sodium 33 ± 2.06 and potassium 22 ± 1.22 (m/l) in sample B. There was no significant difference in the mineral composition in sample A when compared with those of Sample B ($p > 0.05$).

Also, the result of proximate composition revealed that sample A has moisture content of 25%, Ash 4.2%, Crude fibre 10%, lipid 14%, crude protein 2.5%, carbohydrate 38%, 6-shogaol 12.0%, 6-gingerol 15%, paradol 2.5% as well as 22%, 2.6%, 8.5%, 6.5%, 2.5%, 12.6%, 14.5% and 1.5% respectively in sample B as shown in table 2 below. There was no significant difference ($p > 0.05$) in the proximate composition of the two ginger samples.

Table 3 below shows the result of Phytochemical screening of the two ginger samples. The results obtained showed the presence of alkaloids, saponins, tannins, flavonoids, polyphenols and reducing sugar.

The study revealed a significantly increase ($p < 0.05$) the level of survivine, cyclin D1, cyclin B1, cyclin E and and C-MYC proteins in albino rats as shown in figure I below:

Also as shown in figure 2, 3, 4a-e below, there was a significantly decrease ($p < 0.05$) in survivine, cyclin D1, cyclin B1 and C-MYC levels after treatment with increasing concentrations of whole ginger extract especially at 200mg/ml and 250mg/ml respectively.

Table 1: shows the mineral compositions of the two samples of ginger (Ziniber officinael)

MINERALS	COMPOSITIONS	
	Sample A	Sample B
Zinc	4.19 ± 0.60	2.0 ± 0.11
Manganese	28.90 ± 1.60	15.28 ± 0.22
Copper	0.80 ± 0.10	0.94 ± 0.52
Calcium	34.5 ± 1.99	45.0 ± 1.22
Phosphorous	26.30 ± 1.59	28.0 ± 0.52
Iron	6.5 ± 0.88	5.8 ± 0.55
Sodium	38.26 ± 0.44	33.0 ± 2.06
Postassium	36.34 ± 1.94	22 ± 1.22

Sample B = Red Ginger

Table 2: proximate analysis of the two samples of ginger compositions.

NUTRIENT	COMPOSITION	
Moisture	28.10 ± 0.70	22.03 ± 1.66
Ash	6.0 ± 1.22	4.4 ± 2.10
Crude Fibre	14 ± 2.11	12.0 ± 0.91
Lipid	12.0 ± 0.8	6.0 ± 1.10
Crude Protein	2.5 ± 0.32	2.0 ± 0.10
Carbohydrate	38 ± 0.11	36 ± 0.11
6-Gingerol	15 ± 0.22	$18. \pm 0.13$
6-Shogaol	12.0 ± 0.01	14.9 ± 0.11
Paradol	2.5 ± 0.05	1.5 ± 0.02

Table 3: shows the phytochemical compositions of the two ginger samples.

CHEMICAL CONSTITUENTS	METHANOL EXTRACT	
	Sample A	Sample B
Alkaloids	11.6 ± 0.22	13.2 ± 0.2
Saponins	9.20 ± 1.00	7.20 ± 1.66
Tannins	13.2 ± 1.0	11.01 ± 0.41
Flavonoids	8.8 ± 0.2	10.6 ± 0.22
Reducing Sugar	6.2 ± 0.2	9.3 ± 0.32
Polyphenols	21 ± 0.66	18.60 ± 1.11

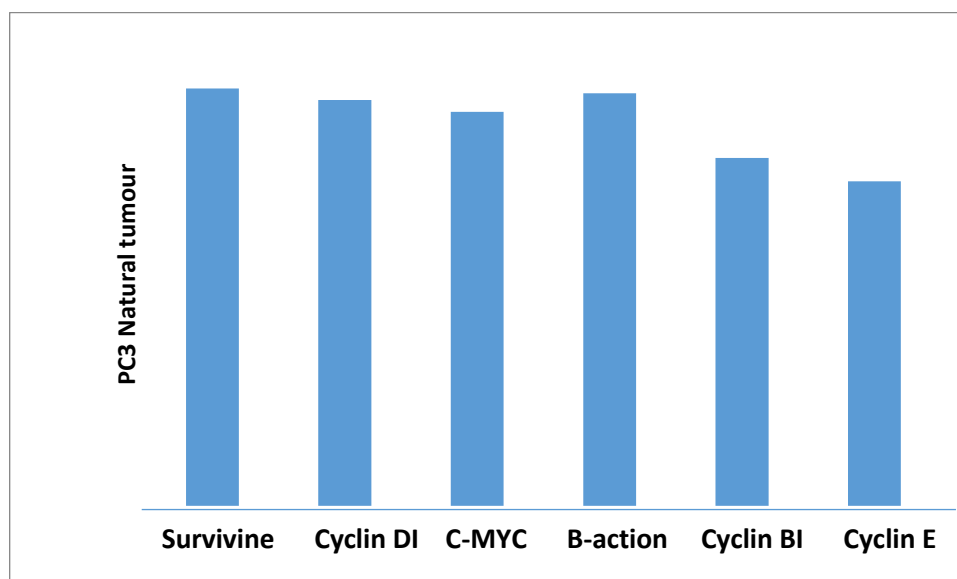


Fig 1: Shows survivine, cyclic D1, B-action and C-MYC proteins in tumour reduce rats with Pc-3 cancer cells.

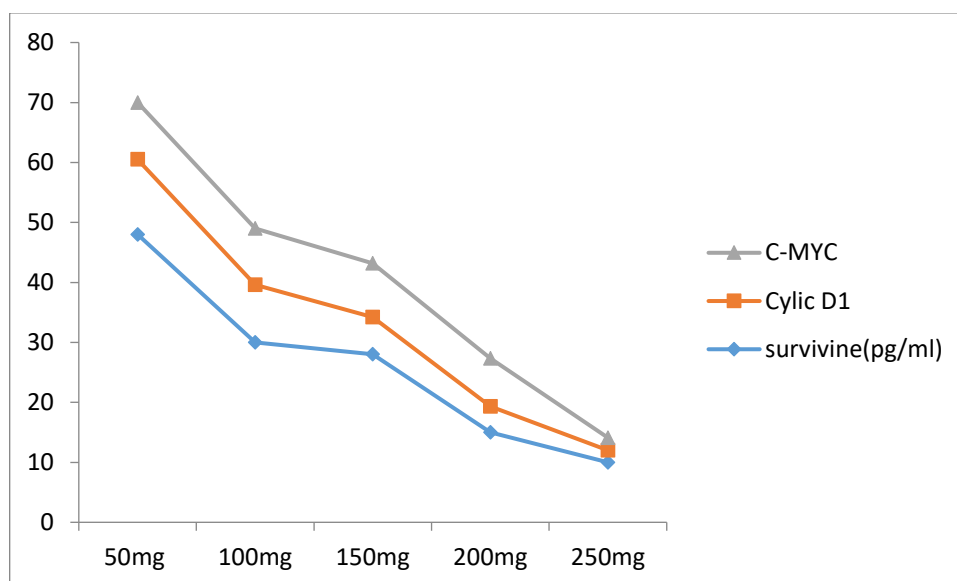


Fig 2: Shows the levels of survivine, cyclin D1, C-MYC in relation to the concentration of ginger extract.

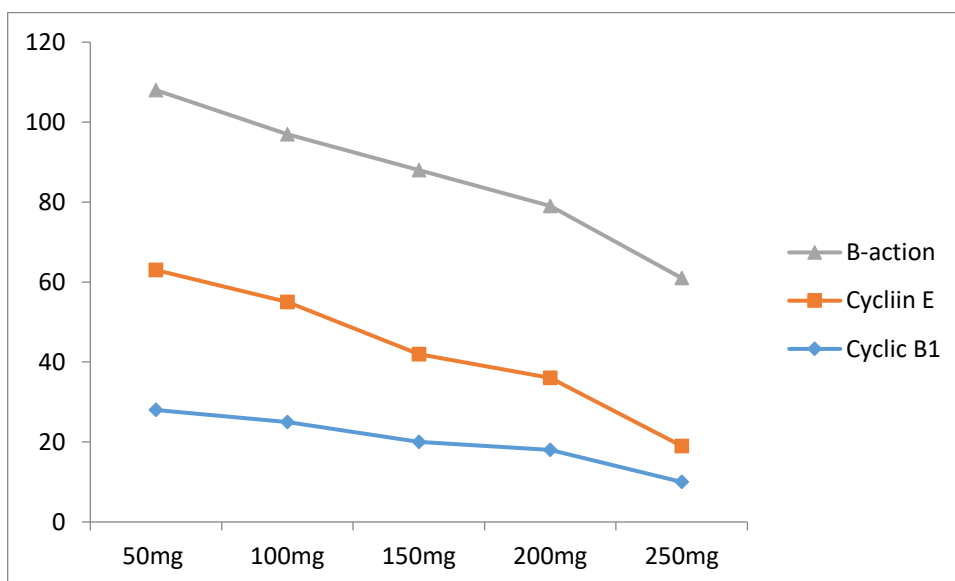


Fig 3: Shows the levels of Cyclin B1, Cyclin E and B-action in relation to increasing concentrations of whole ginger extract.

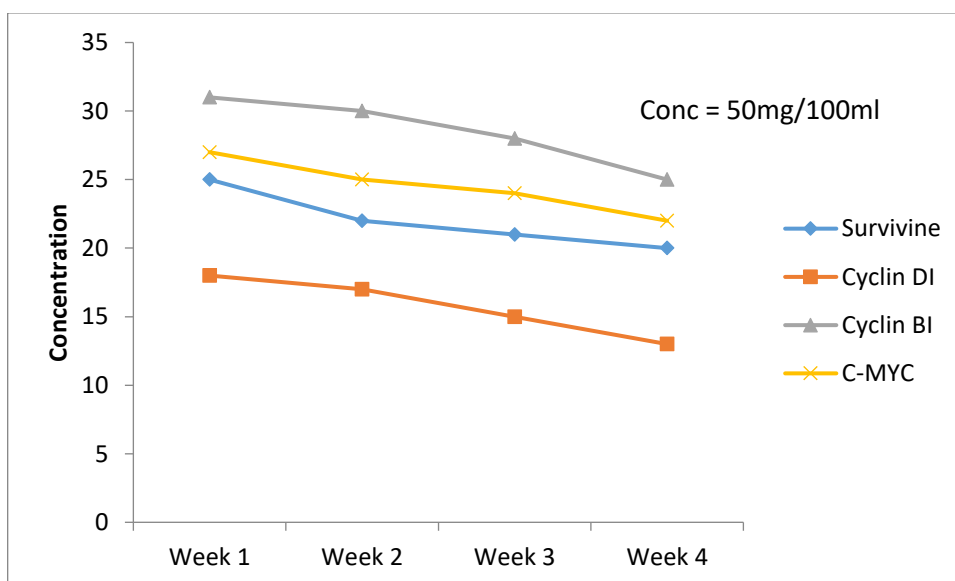


Fig 4a: Effect of concentration of ginger extract in relation to duration of treatment

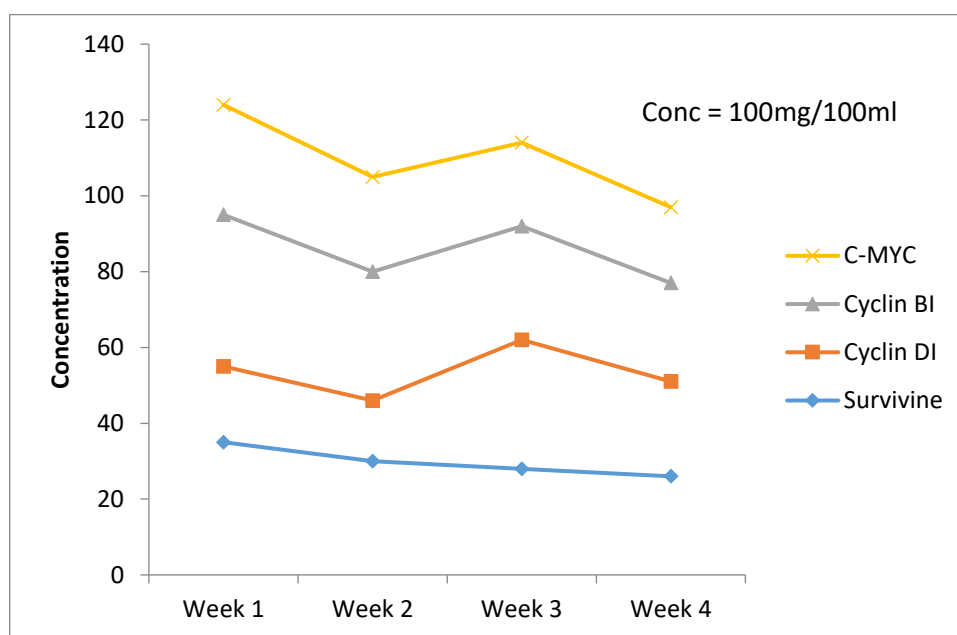


Fig 4b: Effect of concentration of ginger extract in relation to duration of treatment

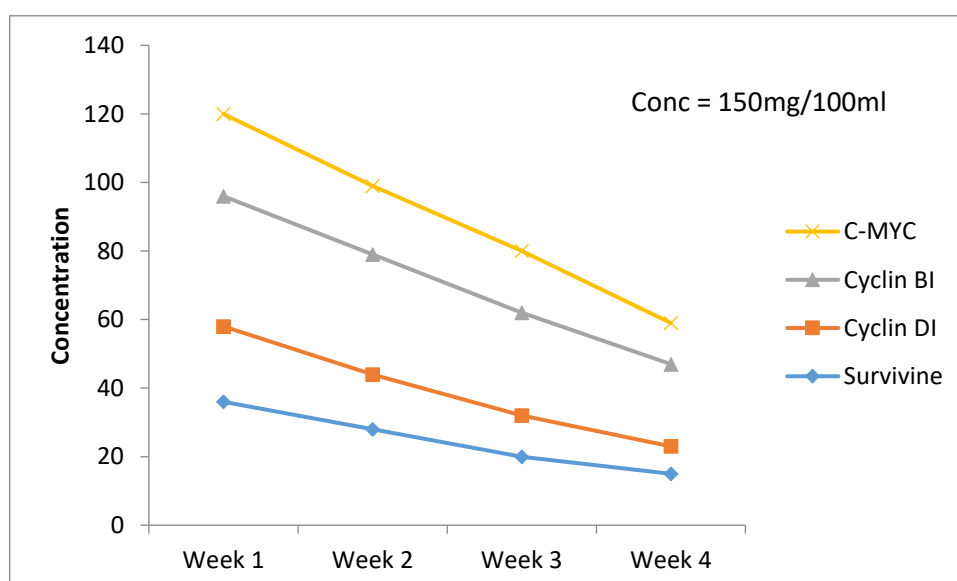


Fig 4c: Effect of concentration of ginger extract in relation to duration of treatment

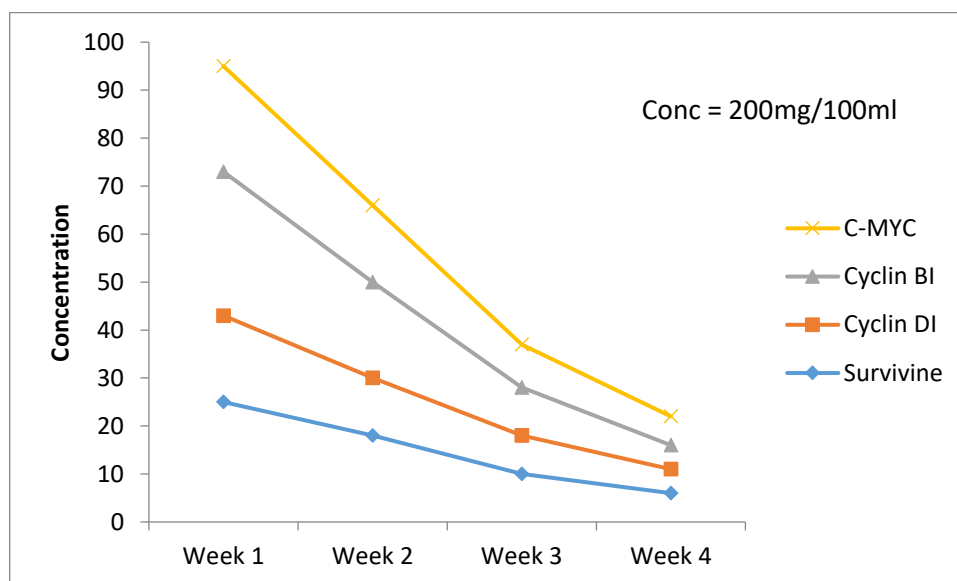


Fig 4d: Effect of concentration of ginger extract in relation to duration of treatment

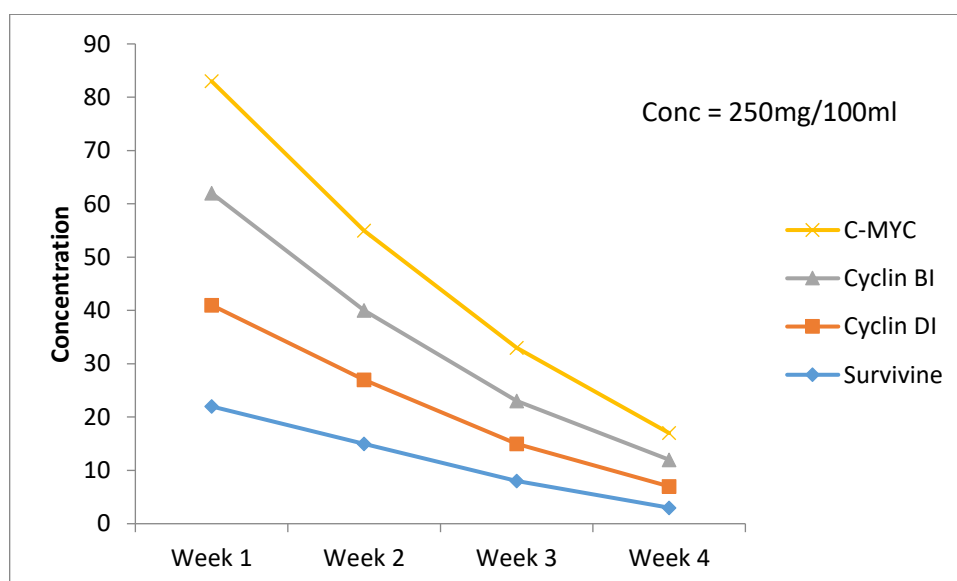


Fig 4e: Effect of concentration of ginger extract in relation to duration of treatment

Discussion

This study was carried out to determine the phytochemical compositions and anti-prostatic cancer potentials of ginger samples grown in parts of Oru East L.G.A., Imo State.

The result of the mineral composition revealed a significant difference in level of zinc 4.19 ± 0.60 , manganese 28.9 ± 1.60 , iron 6.50 ± 0.88 , Sodium 38 ± 2.60 and potassium 36 ± 1.94 in sample A (brown ginger) when compared with those of sample B (red ginger) 2.0 ± 0.1 , 15.28 ± 0.22 , 2.8 ± 0.55 , 33 ± 0.26 and 22 ± 1.22 respectively.

The reported increase in the level of zinc attest to the fact that the ginger sample have some anti-prostate cancer potentials since zinc has been known to promote healthy prostate by way of regulating prostatic hyperplasia and reduces the chances of metastasis in patients with prostate cancer as reported by Folk et al., (2018).

The sodium and potassium levels were found to be normal. This serves as an added advantage since sodium and potassium contribute in compensatory mechanism in cases of electrolytes imbalance in patients with prostate dysfunction.

The calcium levels were significantly high in both samples and were found to be $3.4.5 \pm 0.10$ and $45.0 \pm 0.52\text{mg/lg}$. The reported high level of calcium is important for strong bone formation: This will actually help to take care of oestopores that is very common among patients with prostate cancer.

The iron levels were 6.59 ± 0.85 and $5.8 \pm 0.58\text{mg}/100\text{g}$ respectively for both samples. This shows that the ginger samples could serve as good sources of dietary iron which is very essential in erythropoiesis and maintenance of normal haemoglobin levels in the body. This finding is in consonance with the report of Obodo et al., (2016). They reported iron levels of $9.7\text{mg}/100\text{mg}$ in ginger samples cultivate in parts of South Western Nigeria.

The result of the proximate analysis also shows that the ginger samples have moisture content of $2.8.10 \pm 0.70\%$ and $22.03 \pm 1.66\%$ respectively. This means that the ginger (zingiber officinale) samples can be stored for a long period of time without spoilage.

The ash contents of the ginger samples were recorded as $6.0 \pm 1.20\%$ and $4.4 \pm 2.0\%$, respectively. This result is similar to what was reported by Ferga et al. (2018). They reported ash content of $3.5 \pm 1.11\%$ in ginger samples grown in North Western Nigeria. The ash content is usually a representation of the total inorganic mineral elements in a sample since significant quantity was identified in the samples, it shows that the ginger samples have high mineral elements composition.

The result of crude fibre, lipid, crude proteins and carbohydrate were recorded as 14.0 ± 2.11 , 12.0 ± 0.80 , 2.5 ± 0.32 and 38 ± 0.11 for sample A and 12.0 ± 0.91 , 6.0 ± 1.10 , 2.0 ± 0.10 and 36 ± 0.11 respectively for sample B. The result shows that the ginger samples were good sources of energy required for various biochemicals and metabolic activities in the body.

Also, the result of the study revealed the presence of 6-gingerol (15.02% and 18.60%) 6-shogaol (12.0% and 14.9%) and paradol (2.5% and 1.5%) respectively in the two ginger samples.

These bioactive compounds have been reported by Moore et al (2012) to possess anti-inflammatory and anti-cancer properties.

The result of the phytochemical screening of the zingiber officinale samples revealed the presence of flavonoids. Flavonoids are known antioxidants that promotes healthy blood circulation and strengthens capillary walls. Also, polyphenols, saponins, tannins, and reducing sugar were identified. Polyphenols have been reported by (Folk et al., 2018) to inhibit cancer growth and metastasis.

The study also recorded a significant decrease in survivine, cyclin D1, cyclin B1 and C-MYC proteins in tumor induced albino rats treatment with increasing concentration of whole ginger extract. The reported decrease in cyclin D1, survivine, cyclin B1, cyclin E and C-MYC proteins could be as a result of the presence of 6-shogaol, 6-gingerol and paradol in these ginger samples as reported in the present study.

The cyclin D1, cyclin B1, cyclin E and survivine which can lead to short cancer patient survival, increased metastasis and therapeutic resistance (Moore et al., 2012) were significantly reduced to normal in tumour induced albino rats after treatment with varied concentrations of whole ginger extract.

The present finding is in consonance with the report of Osobo et al., (2018). They reported that 6 – shogaol and 6-gingerol have the potential to decrease the level of STAT 3 and NF-KB regulated target genes at the protein level including cyclin D1 and survivine modulated mRNA levels of chemokines cytokines cell cycle and apoptosis regulatory genes.

Conclusion

The result of the study has provided significant evidence to prove that the ginger samples studied contain high mineral compositions both at micro and macro-levels. These essentials minerals like zinc, calcium, iron, sodium, potassium, copper and manganese are essential in the body especially in fighting and preventing diseases.

Also, the presence of 6-gingerol, 6-shogaol and paradol in these ginger samples further suggests that the samples are good anti-prostate cancer agents, since these compounds have been reported to inhibit the developments and proliferation of prostate cancer cells.

Recommendations

Based on the research findings, we recommend the following:

- Nigerian men should always include ginger as part of their daily diet since it has been proven to contain some bio-active ingredients like shagaol and gingerol which can effectively destroy prostate cancer cells.
- Government should establish clinics for free prostate cancer screening/diagnosis.

Acknowledgement

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Conflict of Interest

The authors have no competing interest.

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