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Phytoconstituent evaluation of Jackfruit and its genotoxic effect using *Allium sativum* assay

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The role of plants by humans as medicine cannot be overemphasized. *Artocarpus heterophyllus* commonly known as jackfruit has been extensively involved in traditional medicines due to the presence of a variety of phenolic compounds. This study aims to further evaluate the chemical composition of *A. heterophyllus* and the possible genotoxic effect of the seeds planted in Nigeria on *Alium sativum.* The chemical analyses of fresh leaves, matured pulps, and seeds were conducted at the National Center for Energy Research and Development (NCERD), University of Nigeria, Nsukka following standard procedures. The genotoxic assay was evaluated using *Allium sativum* bulbs. The proximate concentrations followed the same trend across the leaves, pulp, and seeds (energy > moisture > protein >fibre) except fibre and fat. Vitamin A, B_1, B_2, and B_9 ranged from 1.80 – 2.75, 6.13 – 7.43, 2.20 – 2.70, and 4.30 – 5.17mg/100g respectively while Vitamin C was significantly higher in the pulp (25.83 ± 0.87 mg/100g). Saponin, flavonoids, and tannins were also observed in different concentrations across the parts. The genotoxicity test showed a significant increase in the mitotic index with treatment with 250mg/ml and 500mg/ml extract while all the extract treatments showed higher abnormal dividing cells as compared to the control. *A. heterophyllus* is a valuable source of nutritional and medicinally important compounds. However, due to the proliferative and genotoxic activities observed using the in vivo garlic (*Allium sativum*) root-tip cell test, the extracts of this plant should be studied in animal models to verify its safe use.

Keywords: Allium spp; *Artocarpus heterophyllus;* Genotoxicity; Medicinal Plants; Nutrients; Phytochemicals

INTRODUCTION

Plants are generally used by the human population as medicines, particularly in countries that are developing, where the access to treatment of diseases by conventional medicine is hampered due to the absence of adequate financial resources (Pastori, 2013). *Artocarpus heterophyllus* commonly call jackfruit is a member of the Moraceae and have been extensively involved in traditional medicines due to the presence of a variety of phenolic compounds (Mukherjee, 1993; Wei et al. 2005; Chandrika et al. 2006; Bhat et al. 2017). It has been reported to possess many medicinal properties ranging from anti-bacterial, anti-viral, anti-diabetic, anti-inflammatory immunomodulatory, anti-diuretic, anti-hyperglycemic, to antioxidant (Khan et al. 2003; Wei et al. 2005; Omar et al. 2011; Munira et al. 2015; Bhat et al. 2017). The plant has been useful in the treatment of anemia, asthma, dermatitis, convulsions, cough, constipation, diarrhea, fever, internal heat, skin infections, and snakebite, ulcers, used to increase lactation (Wei et al. 2005; Jagtap et al. 2010). *A. heterophyllus* seeds powder is widely used as an ingredient in the preparation of bakery products, baby foods, beverages, candy, dairy products, instant soups, ice cream, snacks, pasta (Pua et al. 2007; Bhat et al. 2017).

Several kinds of research have discovered that a variety of plants used in traditional medicine or as food have mutagenic effects. These were discovered through cytotoxic and genotoxic evaluations in *in-vitro* and *in-vivo* assays (Higashimoto et al. 1993; Schimmer et al. 1994; Kassie et al. 1996; Celik and Aslanturk, 2007; Celik and Aslanturk, 2010; Pastori, 2013; Costalonga et al. 2017). This has raised great concerns regarding the potential mutagenic or genotoxic hazards that will result from the use of such plants in a long time (Celik and Aslanturk, 2010). The use of many plants containing mutagenic substances had been correlated with a high rate of tumor formation in some human populations (Wynder, et al. 1983; Celik and Aslanturk, 2010).

The use of *Allium* spp L. vegetal system as a bio-indicator of the genotoxic and proliferative capacity of medicinal species had been reported by Bagatini et al. (2009). Besides being a good biological indicator, the evaluation of the chromosomal changes in roots of *A. cepa* had been validated by international safety organizations as an effective test for monitoring the genotoxicity of environmental substances (Cabrera and Rodriguez, 1999; Celik and Aslanturk, 2010). *A. cepa* test is a very good *in-vivo* model that can predict the possible damage to the DNA of eukaryotes which extrapolated for all animal and plant biodiversity. The Allium bulbs test is one method among thefew direct methods used to measure the damage in systems that are exposed to mutagens through the observation of chromosomal alterations (Leme and Marin-Morales, 2009; Celik and Aslanturk, 2010; Tedesco and Launghinghouse, 2012; Ping *et al*. 2012; Leong-on, 2018). This study aims to further evaluate the chemical composition of *A. heterophyllus* and the possible genotoxic effect of the seeds planted in Nigeria on *Allium sativum*.

MATERIALS AND METHODS

Sample collection:

Fresh leaves, matured pulps and seeds of *A. heterophyllus* were obtained from the Botanical Garden of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Chemical Analysis:

The chemical analyses of fresh leaves, matured pulps and seeds were conducted at the National Center for Energy Research and Development (NCERD), University of Nigeria, Nsukka. All chemical content was carried out following standard procedures. Crude protein and fat were conducted using the methods described by Pearson (1976) and Abu *et al*. (2019), moisture, ash, and crude fibre content were determined by the AOAC (1990) methods. The vitamins evaluated were vitamins A, C, B_1, and B_2 following the methods described by Jakkutowicz *et al*. (1997). Flavonoids were also determined according to Bohm and Koupai (1974), Tannin (Pearson, 1976), and saponins (Obadoni and Ochuko, 2001).

Genotoxicity Evaluation

Dried seed samples were ground into powder using a Trans-Wiley Laboratory Mill (Model 4). 200g of the powder was dissolved in 300ml of absolute ethanol, allowed to stay for 24hrs and filtered. The filtrate was air-dried, concentrated in a rotary evaporator, and stored in a refrigerator at 12°C until required. The extract was further prepared into 250mg/ml, 500mg/ml and 750mg/ml concentrations.

Fresh bulbs of *Allium sativum* were grown in water using plastic cups wrapped in black nylon to prevent the penetration of light for about 6 days to ensure proper root formation. They were then transferred into plastic cups containing the different concentrations for 24hrs of the seed extract while water served as a control.

At the end of the treatments, 6 root tips were cut from each garlic bulbs and were washed 2-3 times in tap water and fixed in Carnoy's solution (3:1 absolute ethanol and acetic acid) for 24hours and hydrolyzed with 0.1M HCl at 60°C for 5-7 minutes in a water bath. The slides were prepared following the methods described by Olorunfemi et al. (2015) and Ibeh et al. (2018) accordingly.

Data were collected on cell counts from 3 slides for each concentration. The mitotic index (MI) was determined following Abu et al. (2015) using 500 cells per treatment. The different stages of mitosis and chromosome aberrations were recorded and calculated in 500 cells per slide. Analysis of variance (ANOVA) and Least Significant Difference Test was used to analyze the data obtained.

RESULTS AND DISCUSSION

The proximate composition of the leaves, pulp, and seed of *A. heterophyllus* reveals the nutritional relevance of the plant. The proximate parameters followed the same trend across the different parts (energy > moisture > protein >fibre) except for fibre and fat (Table 1). The moisture content was highest in the pulp and lowest in the seeds. This variation in moisture across the parts was in line with the report of Amidi et al. (2018) on Jackfruits collected from Obiangwu in Ngor-Okpala Local Government Area, Imo State. However, the moisture content in our study was relatively lower than in their report. This variation could be as a result of the difference in the environment and the season of collection. The seeds were significantly rich in energy and fat while the leaves were rich in ash, fibre, and protein. This result agrees with the report of Hettiarachchi et al. (2011) that the seeds of Jackfruits are a good source of dietary fibres and starch. Comparatively, the pulp recorded significantly lower proximate content as compared to the leaves and seeds (Table 1).

The result as presented in Table 2 shows that the leaves, pulp, and seeds contain a considerable amount of vitamins. Vitamin A, B_2, and B_9 were statistically similar across the different parts of *A. heterophyllus.* However, the pulp contained significantly higher Vitamin C while the seeds contained significantly lower Vitamin B_1 (Table 2). Vitamin C is an essential micronutrient required by the body but cannot be synthesized in the body, therefore must be acquired for the external source (Umesh et al. 2010). Vitamin C is cherished for its strong antioxidant properties that protect the body against free radicals, strengthens the immune system, and keeps our gums healthy (Swami et al. 2012).

The presence of phytochemicals in the leaves, pulp, and seeds confirms the usage of the different parts in traditional medicine. Saponins are usually found in tissues that are highly susceptible to attacks by bacteria, fungi, or insects (Wina et al. 2005). Hence, it is assumed that one of the roles saponin plays in plants is to act as chemical barriers in opposition to prospective pathogens. This would, therefore, account for their medicinal uses such as antimicrobial activity, including antispasmodic activity, and toxicity to cancer cells (Osbourn, 2003; Supradip et al. 2010; Ncube et al. 2011; Baht et al. 2017).

The presence of flavonoids further strengthens the use of *A. heterophyllus* parts in traditional medicine. Flavonoids compounds had been isolated from *A. heterophyllus* by Dua et al. (2013) which showed a strong inhibitory effect on lipid peroxidation. Due to the significant nutritional and medicinal properties of this *A. heterophyllus* collected from the University of Nigeria, it will encourage the continuous utilization of the plant, therefore it was pertinent to further evaluate the possible genotoxic effect.

Plant-based bioassays have gained popularity among the toxicological assessment procedures, and some reasons for their wide use are comparative simplicity, sensitivity, and cost-effectiveness as well as a good correlation with other toxicity tests. The results presented showed the average number of dividing cells across the cell division stages (Table 4). *A. heterophyllus* at lower concentration seems to stimulate cell division more than the control. This was observed as the MI was significantly higher with 250mg/ml and 500mg/ml as compared to the control. MI is a useful tool in evaluating the development of the organism exposed to toxins (Costalonga et al. 2017).

Table 1: Proximate composition of the leaves, pulp, and seeds of *A. heterophyllus*

Proximate Parameters (%)	Leaves	Pulp	Seeds
Moisture	58.70 ± 0.68^b	76.78 ± 0.30^a	47.90 ± 2.40^c
Ash	4.12 ± 0.09^a	0.61 ± 0.05^c	1.42 ± 0.03^b
Fat	1.72 ± 0.04^b	1.10 ± 0.25^c	2.67 ± 0.12^a
Fibre	4.63 ± 0.09^a	1.20 ± 0.51^c	3.36 ± 0.21^b
Protein	20.90 ± 0.49^a	6.06 ± 0.19^c	16.21 ± 0.07^b
Energy (kg/KJ)	12352.00 ± 276.66^b	7734.00 ± 105.84^c	17740.33 ± 815.59^a

*****means with different alphabet along each horizontal array represents significant differences

Table 2: Vitamin composition of the leaves, pulp, and seeds of *A. heterophyllus*

Vitamins (mg/100g)	Leaves	Pulp	Seeds
Vitamin A	2.50 ± 0.17[a]	2.75 ± 0.34[a]	1.80 ± 0.12[a]
Vitamin B_1	7.03 ± 0.12[a]	7.43 ± 0.22[a]	6.13 ± 0.09[b]
Vitamin B_2	2.50 ± 0.17[a]	2.70 ± 0.15[a]	2.20 ± 0.12[a]
Vitamin B_9	4.70 ± 0.26[a]	5.17 ± 0.26[a]	4.30 ± 0.21[a]
Vitamin C	20.87 ± 0.70[b]	25.83 ± 0.87[a]	20.00 ± 0.61[b]

*****means with different alphabet along each horizontal array represents significant differences

Table 3: Phytochemical composition of the leaves, pulp, and seeds of *A. heterophyllus*

Phytochemicals (mg/100g)	Leaves	Pulp	Seeds
Saponin	0.08 ± 0.01[a]	0.05 ± 0.00[b]	0.09 ± 0.01[a]
Flavonoids	0.10 ± 0.01[b]	0.25 ± 0.02[a]	0.19 ± 0.02[a]
Tannins	0.34 ± 0.01[a]	0.08 ± 0.00[b]	0.11 ± 0.01[b]

*****means with different alphabet along each horizontal array represents significant differences

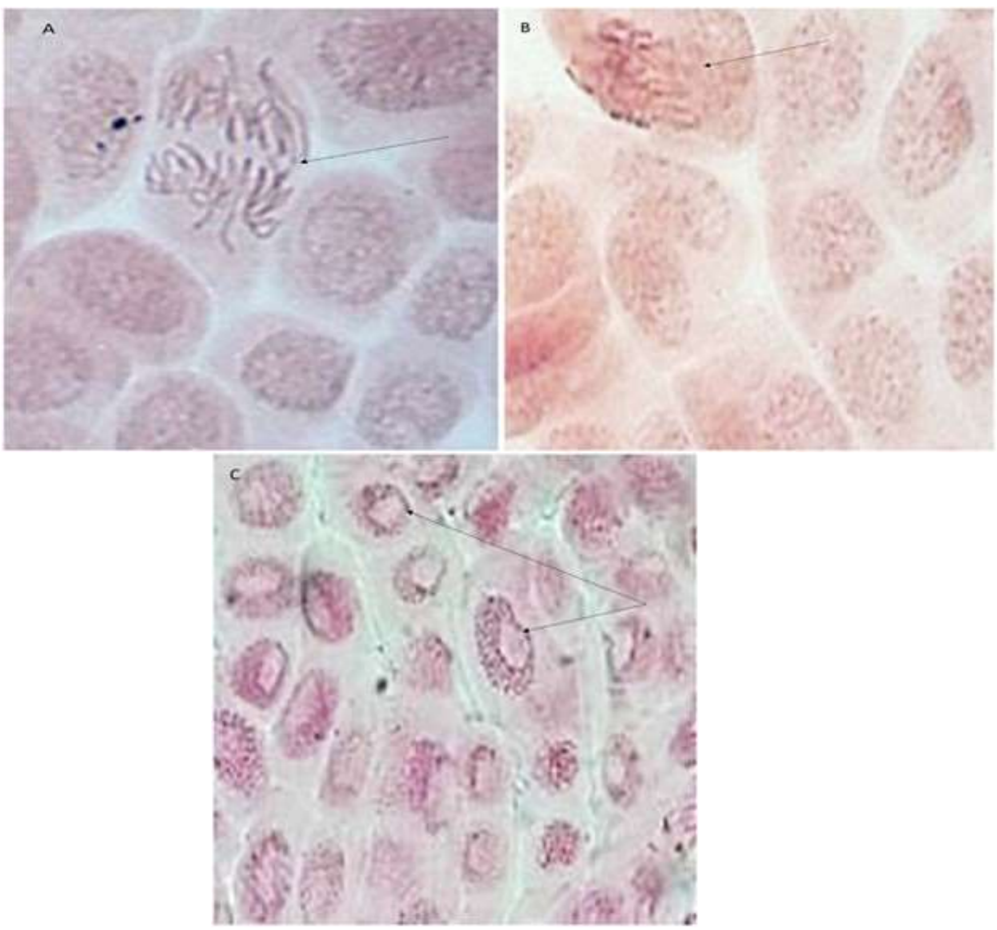

Figure 1: Photomicrographs of the treated *Allium sativum* root tip cells showing different mitotic aberrations

Key: A: Chromosome Bridge at Anaphase, B: Disorganized cell division, C: Vacuolated Prophase.

Table 4: Number of dividing cells at different stages in of *A. sativum* treated with varied concentration of *A. heterophyllus* seed extract

Concentrations (mg/ml)	Prophase	Metaphase	Anaphase	Telophase	*Abnormal dividing cells (%)	*Mitotic Index
0 (Control)	110.75 ± 4.96	79.25 ± 2.17	37.25 ± 1.31	22.75 ± 5.19	5.50 ± 1.50 (2.16)[c]	51.05 ± 1.81[c]
250	126.00 ± 9.88	99.50 ± 6.76	80.50 ± 2.63	35.00 ± 2.80	17.75 ± 1.25 (4.95)[b]	71.75 ± 2.02[a]
500	86.00 ± 5.48	102.25 ± 4.71	30.50 ± 5.58	47.25 ± 2.06	28.50 ± 2.25 (9.77)[a]	58.90 ± 2.23[b]
750	72.75 ± 1.44	53.50 ± 1.94	71.50 ± 6.29	50.25 ± 3.35	19.50 ± 1.44 (7.28)[b]	53.50 ± 1.32[bc]

*****means with different alphabet along each vertical array represents significant differences

The increased MI values suggest an uncontrolled cell division process, which may lead to disordered cell proliferation and tumor development (Souza-Pohren et al. 2013). The increase observed in the significantly higher abnormal dividing cells would be an indication of an uncontrolled cell division process triggered by the extract. However, the seed extract-treated root tips recorded abnormalities less than 10% across concentrations (Table 4). The few abnormalities observed included chromosome bridge at anaphase, disorganized cell division, and vacuolated prophase (Fig. 1).

CONCLUSION

In conclusion, this present study evaluated the significance of *A. heterophyllus* as a valuable source for nutritional and medicinally important compounds portraying its storehouse of proximate, vitamins, and phytochemicals. It provides opportunities for the seed to be used as a functional food. However, due to the proliferative and genotoxic activities observed using the in vivo garlic (*Allium sativum*) root-tip cell test, the extracts of this plant should be studied in animal models to verify its safe use...

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

UDC designed and supervised the experiments and also edited the manuscript. EOO and ACA analyzed the data, wrote the first draft and edited the manuscript. AMP, AOB and OMK conducted the research, managed the literature search and edited the manuscript. All authors read and approved the final version.

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