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IN VITRO TESTING OF METHANOL EXTRACT OF *LUFFA AEGYPTIACA* MILL PLANT (CUCURBITACEAE)

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Abstract

Determination of in-vitro effect of Methanol extract of Luffa aegyptiaca seeds and husk on Tadpoles and Sorghum bicolor radicles has been carried out in this research. The research was carried out to find a local remedy for tumor with the anticipation of possible anticancer constituents. The in-vitro cytotoxicity testing of the methanol extract of L. aegyptiaca seeds and husk on Tadpoles at concentrations of 1, 2, 5, 10, 20 and 30mg/ml and Dimethylsulphuroxide (DMSO) was used as Negative control, the result obtained was 23%, 0%, 10% mortality at 1% concentrations within 27minutes and 76%, 63% and 60% mortality at 24hours respectively. While the in-vitro antiproliferative testing of the seeds and husk of L. aegyptiaca part methanol extract carried out at 1mg/ml, 30 mg/ml also inhibited the growth of Sorghum radicles by 99.2% (husk), 98.7% (seeds) and 97.6% (leaf) at 96 hour.The Luffa methanol extract (LAM) exhibited a concentration defendant cytotoxic effect (LD50 = 18 µg/ml) and (LC50 = 20 µg/ml) inhibition of the growth of guinea corn radicles. The phytochemical screening results revealed that the plant parts contained alkaloid, flavonoids, glycosides and tannins. The results obtained from these studies show that methanol extract of the L. aegyptiaca parts have cytotoxic and antiproliferative effects on Tadpoles and Sorghum radicles. The findings testified the basis for the traditional uses of the plant to treat inflammations and tumor related ailments as practiced in Hausa traditional medicine and suggest the probable use of the plant in preparing recipes for tumor-related ailments.

Introduction

Medicinal plants have been generally recognized to contain phytochemicals or secondary metabolites which perform multiple physiological functions and hence they are believed to have better compatibility with the body (Alagbe, 2023; Singh *et al.,* 2021). The use of plants and their preparations to treat diseases is an age-old practice. Most plants have almost an unlimited capacity to produce substances that attract researchers in quest for new and novel chemotherapeutics (Reed, 2005, Alagbe, 2023, Singh, 2022). Out of the 250,000-500,000 plant species on earth only 1-10% have been studied chemically and pharmacologically for their potential medicinal values (Verpoorte, 2000, Adewale *et al.,* 2021; Singh *et al*, 2021).

The Cucurbitaceae family is vines with tubers, lianas or annual herbs. They are found throughout the tropics and in warm temperate areas. There are 118 genera and 850 species in the family which higher than Momordica with 45 species and Cucurrmis with 52 species. They primarily comprised species which are consumed as food worldwide and are excellent fruit in nature having composition of all the essential constituents for good health of human (Rahman, 2008).

Moreover they provide many plants species with edible fruits, for cultivation among which the best known are cucumber (Cucurrmis), melons (citrullus) and Pumpkins (cucurbita) (Neuwinger, 1996). An intensive investigation in the chemistry, toxicology, biogenesis and genesis of the Cucurbitaceae revealed its bitter principles by South African Research groups, similarly constructed highly toxic triterpene were isolated as bitter principles from 99 of 850 Cucurbitaceae species that is the so-called Cucurbitacins (A-R) they occur in the plant as glycoside.

Cucurbitacins are group of diverse highly oxygenated triterpenoids molecules. Quite interestingly, Cucurbitacins have shown antiproliferative effects on other cancer cell lines (Escandell *et al,* 2008). Cucurbitacins were isolated from plant family such as Cucurbitaceae, crucifereae that have been reported to have several bioactivities used for centuries as folk medicines (Chen *et al.,* 2005, Alagbe, 2019; Farag *et al*., 2015). A large number of biological activities have been attributed to cucurbitacins and to their glycosylated derivatives (Saba *et al.,* 2010). Phytochemical constituents reported to be present in abundance within the family members are Alkaloid, Cardiac glycoside, terpenoids, Saponins, Tannins, and other various phenolic

derivatives (Mohammed M. *et al* 2019). They are originally isolated from Cucurbitaceae plants such as; *Ecballium elaterium*, *Citrillus colocynthes* or *Cucurbita pepo*, *Cayaponia tayuya*, *Trichosanthes kirilowii* (Tannin-Splitz *et al.*, 2007).

The representatives of the Cucurbitaceae were important in traditional medicine of the Greeks, the mediaeval doctors valued their healing properties and even now there are several species in the Pharmacopoeia (Neuwinger, 1996). They contain large range of secondary metabolites belonging to different chemical families and possess distinct biological activities (Rizvi *el al.*, 2009, Amin *et al*., 2009). Terrestrially the fruits of *Citrullus* and *Acanthopsiceae* are the most important water providers of the Kalahari bush people of Botswana; 70-80% of the weight of melon is water. Cucurbitacins predominantly found in the family Cucurbitaceae, having great interest because of the wide range of biological activities like; antioxidant, anti-inflammatory and inhibition of the proliferation of cancer cells (Tannin-splitz *et al.*, 2007).

MATERIALS AND METHODS

Collection and Preparation of *L. aegyptiaca* plant parts

The seeds and husks (dry fruits) of the plant were collected in March 2011, in Zaria, Kaduna State. The plant's identity was confirmed by Taxonomist Mal. U.S. Gallah of Department of Biological Sciences, Ahmadu Bello University, Zaria, with the specimen voucher number 365 and was deposited at the Herbarium Unit of the Department. The plant parts were dried under shade, in the laboratory at room temperature for a week, and then powdered, using pestle and mortar and powdered plant parts were kept in a container until required for future use.

Extraction of the *L. aegyptiaca* Plant Materials

Powdered seeds and husks 300g each of the plant were separately macerated with occasional shaking in 2 liters of methanol for 24h; they were filtrated and the filtrates were concentrated in a water bath at a boiling point of 48^{o}C to 50^{o}C.

Sources and identification of tadpoles of *Raniceps ranninus*

Tadpoles of *Raniceps ranninus* were obtained from toad colonies in pond around the Faculty of Pharmacy, University of Benin, Benin city, Nigeria. They were identified and confirmed by an Animal Parasitologist Prof. M.I.M. Aisien, Department of Animal and Environmental Biology, Faculty of Sciences, University of Benin, Benin city, Nigeria.

Source and Preparation of the *Sorghum bicolor*

Sorghum bicolor obtained in February, 2011 from Samaru market, Samaru-Zaria, Kaduna State, Nigeria was cleansed with absolute alcohol after which the seeds were dried before use. The viability of the seeds were determined by placement in a water container; those that were able to remain submerged in water were selected and dried for use, while those that float were discarded (Ayinde *et al.*, 2011).

Determination of cytotoxic effects of *L. aegyptiaca* methanol extracts against *Raniceps ranninus*.

This was achieved by placing 10 tadpoles (5-6 days old) in 250 ml capacity beakers containing 30 ml of the water from the source (environment) of the tadpoles, which was made up to 49 ml with distilled water, the volume were made up to 50 ml with; 1 mg/ml, 2 mg/ml 5 mg/ml, 10 mg/ml, 20 mg/ml and 30mg/ml of the extract dissolved in 5% Dimethylsulphuroxide (DMSO) in water, thereby making concentrations of 20 µg/ml, 40 µg/ml, 100 µg/ml, 200 µg/ml, 400 µg/ml, and 600µg/ml respectively.

The experiment was carried out in triplicates. Negative controls were setup for the experiment in which the tadpoles number and medium was made similar only that there were no methanol extract added in the control but Dimethylsulphuroxide. The mortality rates of the tadpoles were observed for a maximum of 24hrs that was indicated by their inability to be active and their death was confirmed when they remain immobile submerged and turns upside down (Ayinde and Agbakwuru, 2010).

Determination of growth inhibitory effects of *L. aegyptiaca* methanol extracts on *S. bicolor*

10 ml of 1 mg/ml, 2 mg/ml, 5 mg/ml 10 mg/ml, 20 mg/ml and 30 mg/ml the methanol extracts were dissolved in 5% Dimethylsolfoxide (DMSO) in water and were poured separately in to 9 cm-wide Petri dishes under laid with cotton wool and a Whatman No1(filter paper).

Twenty (20) viable seeds were spread on each plate and incubated at dark environment. The negative controls were treated with 10ml of 5% Dimethylsolfoxide (DMSO) in distilled water only. The lengths (mm) of the radicles emerging from the seeds were taken at 24, 48, 72, and 96 hours. The experiments were carried out in triplicates (Ayinde *et al.*, 2011)

RESULTS

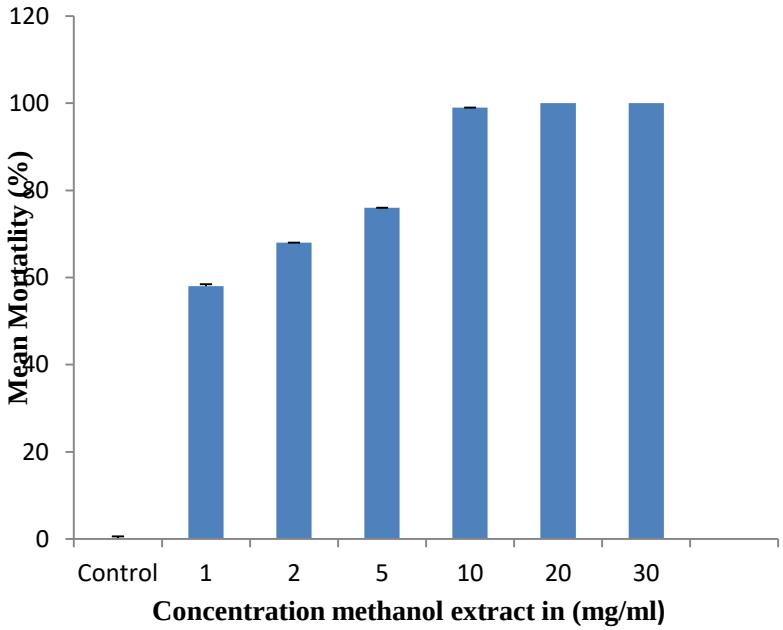

Fig: 1 Cytotoxic effects of the methanol extracts of *L. aegyptiaca* husk on Tadpoles.

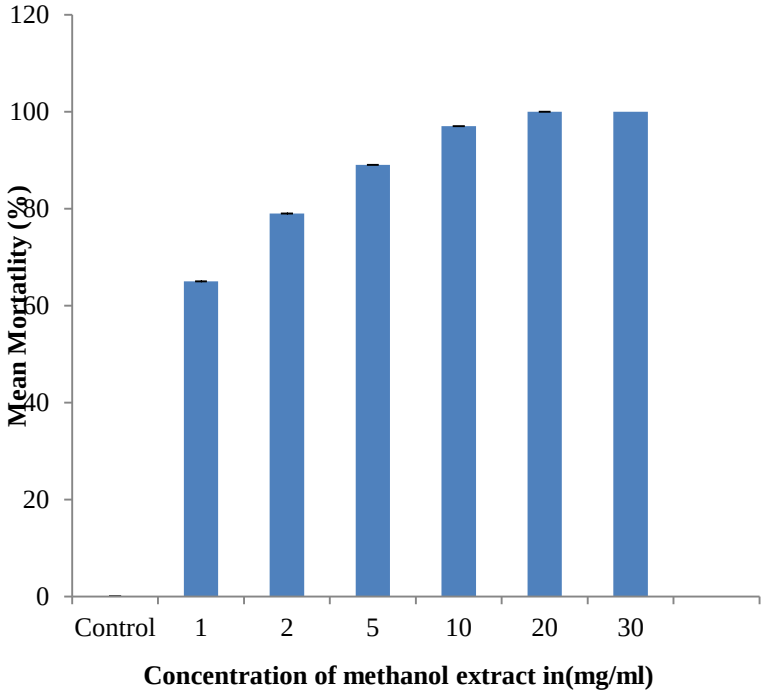

Fig: 2 Cytotoxic effects of the methanol extracts of *L. aegyptiaca* seed on Tadpoles

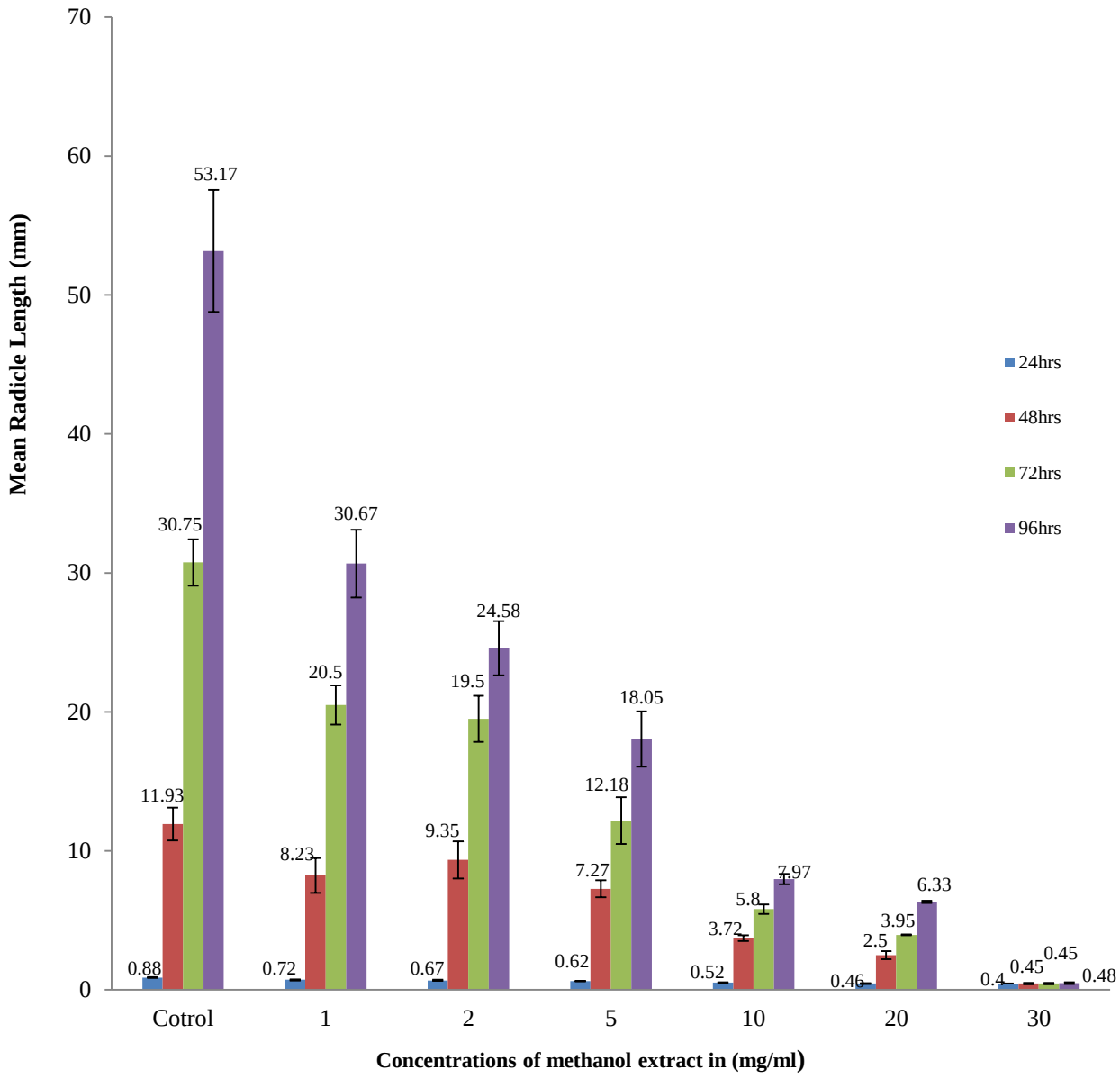

Fig: 4 Antiproliperative effect of *L. aegyptiaca* husk methanol extract on *S. bicolour* seed radicles

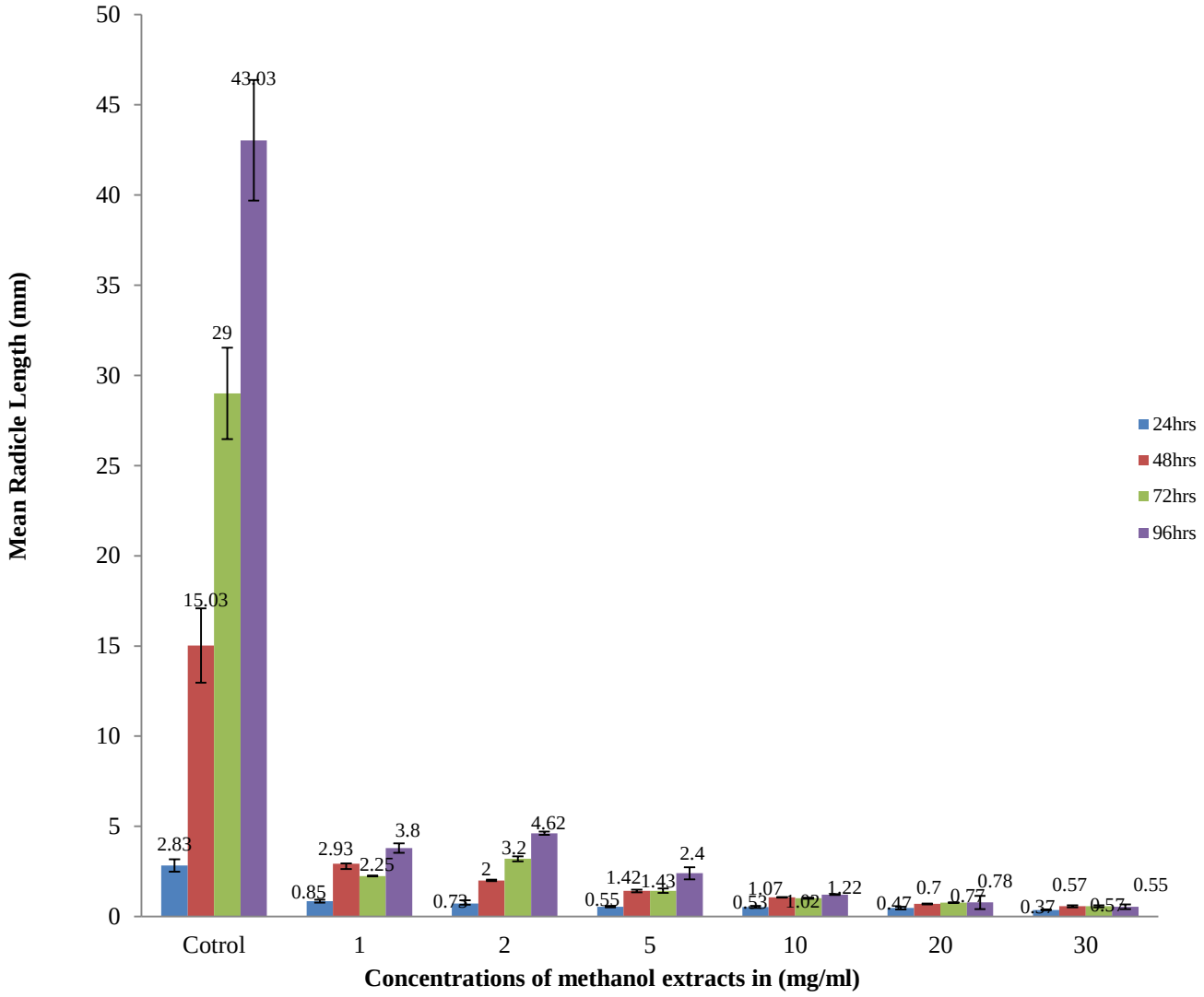

Fig: 5 Antiproliperative effect of *L. aegyptiaca* seeds methanol extract on *S. bicolour* seed radicles.

DICUSSION AND CONCLUSION

Cancer is a broad term used to encompass several malignant diseases ravaging different parts of the body. The hallmark of cancer is uncontrolled cellular growth with subsequent cell invasion and organ metastasis which is the major causes of most cancer related mortality (Koledoye O, *et al* 2021). Therefore the uncontrolled multiplication of the cell leads to the production of tumor cells; this can be linked with the rapid growth and multiplication of exhibited by the meristematic cell of a germinating seeds or a growing of seed radicals. Research in to anti-tumor

agents usually involves a series of complex procedures that sometimes produce non encouraging results after much of materials and time has been expended, in view of these, paucity of research funds has made the development and acceptance of simple bench-top assay very imperative(Malaughlin JL, *et al* 1991).

Several bench-top assay have been used to investigate the ability of plant extract to impart cytotoxicity on certain organisms like; the mosquito larvae, nauplii of Artemesia salina, brine shrimp and Tadpoles (Gerai S. *et al* 2018). The method provide an encouraging, producibility results, advantageously it is simple and can be used to screen medicinal plants with probable anti-tumor effects (Ayinde *et al* 2010). The used of mosquito larvae, nauplii of Artemisia Salina has been reported to be a measure of the plant extract to inhibit growth of tumor-producing cells, induced dormancy in plant seeds or allelophathic effects (Malaughlin JL, *et al* 1991).

The availability of the tadpoles particularly in the raining season encourages their usage in this work, even though it could be a limiting factor carrying out this type of work in dry season or in the areas where depth and saline waters. The result obtained in the antiproliferative effects, reduced in the radicle length of the seeds suggested the probable application of the plant materials at high concentrations/doses as herbicidal. It could be anti-tumor or allelopathic as it possibly interfering with multiplication of the meristematic cell growth related system, such as DNA division of the seed radicals hence reduced/inhibits the normal growth compared with to the control.

The fact that the extract of the leaf at low concentration displayed higher lethality or cytotoxicity on the tadpoles compare to the inhibitory effect of the radical length at the same concentration, it is an indication that the two organisms varied in their physiological compositions hence lead to the differences in the extracts activities.

Luffa aegyptiaca like other plants in the Cucurbitaceae family produces many seeds, and this in some other way round, may account for the dominant nature of the plant family on farmland or in the wild, there is a tendency that this property is as a result of the secretions of certain chemical constituents in to the surrounding soil which may deter or stunted the growth and germination of other plants around it, that is allelopathic influence which may lead to possible formulation of herbicides.

Phytochemical constituents reported to be present in abundance within the family members are Alkaloid, Cardiac glycoside, terpenoids, Saponins, Tannins, and other various phenolic derivatives (Mohammed M. *et al* 2019). GC-MS analysis of *Luffa aegyptiaca* ethanolic leaf extract shows that it contains 35 bioactive compounds with marked pharmaceutical properties (Alagbe JO, *et al* 2023). The activity of the extract is observed to be higher on tadpoles lethality than the inhibitory effect on the growth of seed radicles. On the tadpole this assertion was indicated by the EC_{50} of 18µg/ml and EC_{50} of 30 µg/ml in leaf and seeds extracts respectively when compared with the control.

The triterpenoid, steroids, Cucurbitacins presents as a taxo chemical marker for the family Cucurbitaceae, was reported for its antiproliferative effects by mediating through suppressing of phosphotryosine of signal transducer Activator of transcription3 (STAT3) signaling pathway (Smith *at el* 2000). Same Cucurbitaceae was reported to show suppressed skin carcinogenesis (Konoshima *et al* 1995) and it inhibits cell adhesion (Musza *et al* 1994).

Cucurbitacin-R also reported present in *L. aegyptiaca* found to be one of the allelopathic constituents of *Cayaponia tayuya* inhibit the anti-inflammatory and analgesic properties by Cucurbitacin; 23, 24-dihydrocucurbitacins B (DHCB) and Cucurbitacin-R (CCR) they demonstrated that, the anti-inflammatory, anti-analgesic and anti-arthritic activity of DHCB and CCR in-vitro and in-vivo was due to the ability to inhibit the expression of TNF∞ in lymphocyte and in monophages and their interference with the activity of the Nuclear factor NF-AT. These suggested the probable presence of the major constituents responsible for these activities to be more concentrated in the leaf than in the seeds. *Legenaria breviflora*, *Cayaponia tayuya*, *Luffa cylindrica*, *Cucurbita andreana* of Cucurbitaceae family have been reported to potently exact antiturmorigenic activity in the absence of activated STAT 3 (Saba A.B *et al* 2010).

Other antiproliferative effects of this family was reported for their abnormal action in activation of STAT 3 is prevalent in breast, pancreatic, ovarian head and neck, brain and prostate carcinomas, as in melanomas leukemias and lymphomas (Saba Adebowale *et al* 2010).

The family (Cucurbitaceae) was reported to possessed abundant oxygen species such as hydroxyl radical, superoxide anion radicals and singlet oxygen and other Carbon/nitrogen reactive species hence became antioxidant, these properties may cause them to attack protein, polyunsarated. fatty acid in cell membrane (given rise to lipid peroxidation) or DNA, causing the alteration of gene expression and cellular metabolism.

Antioxidants are of four category base on their mechanism of action as: Preventive, Radical scavenging, Repairs and de novo, then the adaptive antioxidants, Naguchi *et al* 1994, referred that found in the cucurbits to possess preventive and radical scavenging antioxidant properties. Blaskovich *et al* 2003, observed that Cucurbits contains the constituents the suppressed the level of Phosphotyroxine STAT-3 in V-Src-transformed NHI 3T3 cells and human cancer cells potently (IC_{50} value of 500nm in the human lung adenocarcinoma A549) and rapidly (complete inhibition within 1-2 hrs). Siqueira *et al* 2007, back the above assertion based on the action of *Cucurbita andreana* fruit extract which inhibited cyclooxygenase-2 (COX-2) –enzymes and had no side effects on COX-1 enzymes. Another major phytoconstituent of Various Pharmacological activities of LemLE, saponin and its aglycone sapogenin have been reported ranging from immunostimulant, antiproliferation, antiangiogenesis and apoptotic effects (Parama *et al* 2020).

CONCLUSION

The result obtained in the effects, reduced in the radicle length of the seeds suggested the probable application of the plant materials at high concentrations/doses as herbicidal. While the Cytotoxic, it could be anti-tumor or allelopathic as it possibly interfering with multiplication of the meristematic cell growth related system, such as DNA division of the seed radicals hence reduced/inhibits the normal growth if compared with the control.

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