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Glial Fibrillary Acidic Protein Expression and Histopathology of Rat's Cerebrum Following Consumption of Ethanolic Stem Extract and Juice of *Costus afer*

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Authors' contributions

This work was carried out in collaboration between all authors. Author AAO designed the experiment and carried out the laboratory work. Author EE procured the experimental animals and was involved in extract administration. Author EMA did the statistical analysis. Author ITE handled photomicrography and author IOA took care of the extraction process. Author ETB was the supervisor and adviser on scientific procedures and methodology. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Costus afer (C. afer)* is a medicinal plant commonly used as a herbal remedy for diabetes and hypertension in our country, Nigeria The aim of this study was to evaluate the effect of crude ethanolic stem extract/juice of *C. afer* (bush cane) on the cerebrum of rats.
Study Design: This study is designed to test possible adverse effect of *C. afer* stem extract and

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stem juice on the cerebral histology and astrocytic protein, glial fibrillary acidic protein expression using the rat's model.
Place and Duration of Study: This study was carried out in the department of Human Anatomy, Faculty of Basic Medical Sciences, University of Calabar, Nigeria between May and July 2015.
Methodology: Adult albino Wistar rats weighing between 150 g – 210 g were divided into four groups of six rats each. Group I served as the control group and received distilled water, Group II was given the extract, 200 mg/kg weight of rat. Group III, was given the extract, 500 mg/kg weight of the rat while group IV was given the juice, 5ml/kg weight of the rat. Administration was done orally once a day using the orogastric tube for 28 days, at the end of which the animals were sacrificed g chloroform anaesthesia and preserved in buffered formaldehyde. The gross morphology was observed, histology of the cerebrum was studied using Haematoxylin and Eosin stain, and glial fibrillary acidic protein as an index of brain injury was assessed using immunohistochemical procedures.
Results: Results showed that *C. afer* had no adverse morphological effect on the rats. A mild vacuolation of the cells and reactive astrogliosis were observed on sections of cerebrum from the experimental animals. These indicate toxic effects on the cerebrum of Wistar rats
Conclusion: It was concluded that *C. afer* stem extract and juice administered to rats caused vacuolation of cells and astrogliosis in the cerebrum and these indicate toxic effect.

Keywords: Costus afer: stem extract; juice; cerebrum.

ABBREVIATIONS

C. afer- Costus afer; ml- mililitres; kg-kilogramme; H & E- Haemtoxylin and Eosin.

1. INTRODUCTION

In many countries including Nigeria many indigenous plants are used as spice, food or medicine [1]. These plants often exhibit a wide range of biological and pharmacological activities. Extracts from the roots, barks, seeds and fruits of these plants are used in herbal preparations in traditional medicine as cough suppressant and in the treatment of oxidative related disease [2]. It is generally assumed that the bioactive substances contributing to these protective effects are the phytochemical constituents, vitamins and minerals [2]. Various organic compounds derived from plants are important in combating different diseases. Knowledge of these phytochemicals and their specific Pharmacological activities will go a long way in the management of some disease conditions [3].

The plant *Costus afer* is a tall perennial herbaceous medicinal plant with creeping rhizome commonly found in most shady forest and river banks of tropical West Africa [4]. *C. afer* is commonly called ginger lily or bush cane. It is known as *ukpete* or *okpoto*" in Igbo land, "*Kakizuwa*" in Hausa and *tete-Ogun* in Yoruba, "*mbritem*" in Efik and "*ogboduo*" in Ijaw in Nigeria. Anglophone countries call it "monkey Sugar cane." It is commonly used as a medicinal herb especially the leaf. Stem, seeds and rhizomes which are harvested from the wild bush are also used [5]. It has been used in folkloric medicine to treat ailments such as inflammation, rheumatism, arthritis, cough, hepatic disorders, helminthic infection, miscarriage, epileptic attacks and hemorrhoids. It also serves as laxative, diuretic and as an antidote for poison [6,7].

C. afer is a useful medicinal plant that is highly valued for its anti-diabetic [8-10]. The effect of aqueous stem extract of *C. afer* in diabetic induced rats showed that the islet of langerhan of the pancreas were increased in number in all groups treated with the plant extract, against diabetic control group in which the islet cells were reduced [9].

It has been reported to have antinociceptive property [11], anti-inflammatory [12] and anti arthritic properties in South East and South-West Nigeria. The tea from the dried aerial part is used against hypertension while the leaves are used as poultry feed additives to increase both the size and number of eggs of treated birds [13]. The leaves are reported to be an effective remedy for fever and malaria when boiled with leaves of *Carica papaya* (pawpaw), Citrus species (orange) and bark of *Magnifera indica* (mango). The leaf is also used for traditional therapy against measles, malaria, eye defects, hunch-back and evil repellants [14]. The stem

and juice have traditional use for treatment of cough, measles and malaria. The roots mashed to a thick paste are applied topically to abscesses and ulcers. Stem decoctions mixed with sugar cane juice are used to treat cough, respiratory problem and sore throat. The smoke of dried stem is also inhaled to treat cough. Aqueous extract of *C. afer* was used against gentamicin induced nephrotoxicity in rats and was found to decrease the serum sodium, blood urea and creatinine level and increased serum potassium level. Authors concluded that aqueous extract of *C. afer* may attenuate gentamicine induced nephrotoxicity in rats [15]. The methanolic extract of *C. afer* stem has a hepatoprotective and antioxidant activity [16]. The hypolipidemic activity of the aqueous extract in rats was also reported. The significant lipid lowering activity in diet induced hyperlipidemia suggest a beneficial role in the treatment of cardiovascular diseases [17]. *C. afer* ethanolic leaf extract has been reported to have antimicrobial activity against gram-positive and gram negative bacteria except against *Klebsiella pneumonia* where it was 100% resistant [18].

The phytochemical, mineral element and anti-nutrient analysis carried out on the leaf/stem of *Costus afer* showed that it contains saponin, alkaloid, glycoside, tannin, steroid and terpenoids [15,19,20]. Also proximate mineral element and antinutrient analysis showed that the leaf of *Costus afer* contains moisture, crude fat, crude protein, carbohydrate, crude fibre and ash. The mineral composition includes calcium, magnesium, potassium, sodium and phosphorus. The antinutrient include; oxalate, cyanide and tannins. The presence of phytochemicals in the plant coupled with supposed low toxicity level obtained contributes to the beneficial role of *C. afer* in folk medicine [21].

The aim of this study was to assess possible toxic effect of *C. afer* ethanolic stem extract and juice on the cerebral histology and GFAP expression in adult Wistar rats since the extract is widely consumed and scientific reports on its possible side effects on the histology of the cerebrum and glial fibrillary acidic protein is lacking.

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Extract

Fresh stem of *Costus afer* were obtained from the Botanical farm of the University of Calabar and identified by a taxonomist Effa Effa A. A sample specimen was deposited with voucher number 559 in University of Calabar. The stems were washed, cut into pieces and air dried for two weeks. The dried pieces were homogenized into powder form using an electric blender. The powdered plant material, 7 kg was obtained after grinding. The powder was then socked in a plastic bucket in an equal volume of ethanol and dichloromethane (1:1). The solvent to solute ratio being 2:1, it was soaked for 72 hours for thorough extraction of active components. The mixture was filtered first using a chess cloth followed by the filtrate being filtered through watt man No. 1 filter paper of pore size 0.45 micrometer. The filtrate was placed in beakers and allowed to concentrate in water bath by evaporation at 40℃ to complete dryness yielding the extract.

2.2 Extraction of *Costus afer* Stem Juice (CASJ)

The collected stems were debarked after that the foliage leaves covering them were removed. The debarked stems were then weighed and put into a clean manual blender where they were crushed to squeeze out their juice. The resulting paste was filtered to obtain 10mls of the CASJ each day.

2.3 Animals and Experimental Procedures

Twenty six male and female albino Wistar rats weighing between 150 g to 210 g were purchased from zoology animal House, University of Calabar. They were kept in the animal house of the Department of Human Anatomy, College of Basic Medical Sciences, University of Calabar. The animals were kept in a standard room temperature of about 25-27^{o}C throughout the duration of the experiment. The weights of the rats were taken before commencement of the experiment and at intervals throughout the period.

The rats were divided into four groups (Control, High dose, Low dose and Juice) of six animals each. The extract was administered through oral route using orogastric intubation at a dose of 200 mg /kg body weight for low dose, 500 mg /kg body weight for high dose and 5 ml/kg body weight of the stem juice. The control group received equal amount in volume of distilled water as the extract. Administration was done once in a day for 28 days. At the end of the

administration (i.e. after 28 days), the animals were sacrificed using chloroform anaesthesia and perfussed with 10% buffered formaldehyde to flush out the Blood. The organs were dissected out and preserved immediately in the10% buffered formaldehyde after which they were processed for Haematoxylin and Eosin staining process [22] as well as immunohistochemical assessment for Glial fibrillary acidic protein [23].

3. RESULTS

Morphologically, there was no observable morphological disorder in the rats after administration of the extract.

3.1 Histologically

The following were observed.

Control group: Sections of the cerebrum from the control animals had normal histological features of the cerebrum. Only three layers were distinguished in this experiment - :Marginal layer, the Cortical plate, Subcortical plate designated as layers I, II and III. The neuronal cell bodies, 'N' were prominent in the control sections (Fig. 1). In the test group administered with 200 mg /kg, sections of the cerebrum showed normal histological features with only three layers and different cell types when compared with the control (Fig. 2). In the test group administered with 500 mg/kgw. sections of cerebrum showed some cells with vacuoles 'V' (Fig. 3). Sections of the cerebrum from test animals administered with 5 ml/kg weight of the animal had cells with vacuolations compared with control (Fig. 4). Imunohistochemically, Sections of cerebral cortex from test animals showed increasing positive expression of Glial fibriillary acidic protein (GFAP). This was indicated by the increase in reactive astrocytes which increased with increasing dose and was observed more in the test animals that received *C. afer* stem Juice group (Figs. 5- 8).

4. DISCUSSION

Medicinal plants play a great role in human life and contains bioactive substances that are useful for traditional therapeutic application and modern drugs production. *C. afer* as a medicinal plant is commonly used for traditional health and other socio-cultural purposes [14].

Our study revealed no significant effect of extract treatment on the body weight of the experimental animals compared with the animals in the control group. This is different from the report from other authors [24] in which they observed a significant change in weight of the rats after treatment for a period of 56 days. The extract of *C. afer* has been reported to have hypolipidemic property [17]. One would have thought that the animals would lose weight during the period of treatment but this was not the case in this study. Extract of *C. afer* may contain some constituents with pharmacological properties which effect is useful to health.

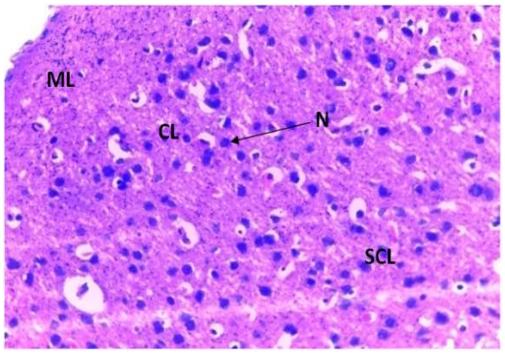

Fig. 1. Photomicrographs of the cerebrum from the control rat

Section of the cerebral cortex showing three distinct layers : The marginal layer(ML), Cortical layer(CL), Sub cortical layer (SCL). The neuronal cell bodies (N) are prominent with pyramidal to round shaped deeply strained cells. The cells within the granular cell layers are densely packed. (H & E; Mag. X160)

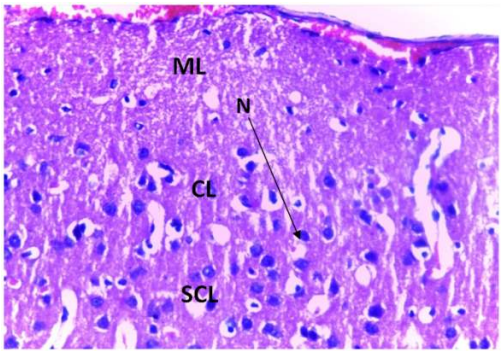

Fig. 2. Photomicrographs of the cerebrum from rats tested with ethanolic stem extract of *Costus afer*

Animals in this group received the extract, 200mg /kg body weight of rat. Section shows distinct cerebral cortex with the layers intact. The neuronal cell bodies (N) are prominent with regular cytoplasmic and nuclei outline. (H & E; Mag.X160)

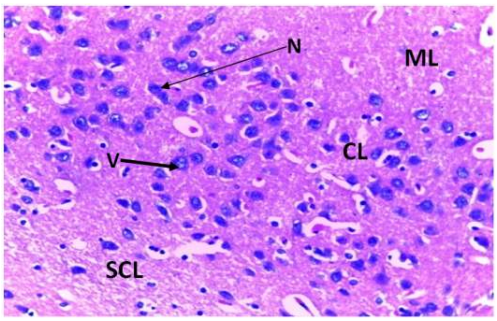

Fig. 3. Photomicrographs of the cerebrum from rats tested with ethanolic stem extract of *C. afer*

*Animals in this group received extract, 500 mg /kg weight of rat. Sections (plate 3) show distinct cerebral cortex with the layers. The neuronal cell bodies (N) are prominent with regular cytoplasmic and nuclei outline. Mild Cell vacuolation(**V**) observed (H & E; Mag. X160)*

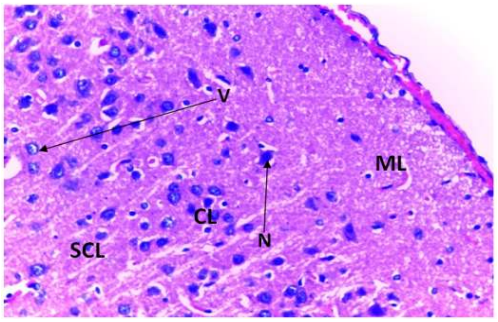

Fig. 4. Photomicrographs of the cerebrum from rats tested with *C. afer* stem juice

Animals in this group received the stem juice, 500ml /kg body weight of the rat. Section shows distinct cerebral cortex with the layers. The neuronal cell bodies (N) are prominent with regular cytoplasmic and nuclei outline. (H & E stain; Mag. X160). Vacuolation(V) of cells was observed

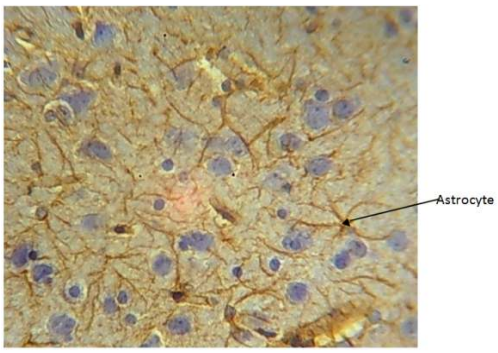

Fig. 5. Photomicrograph of cerebrum from control rats

Shows Glial Fibrillary Acidic Protein (GFAP) expression in astrocytes of rat's cerebrum. (Brown cells with processes indicate astrocytes). Immunohistochemical stain (IHC) (X 400)

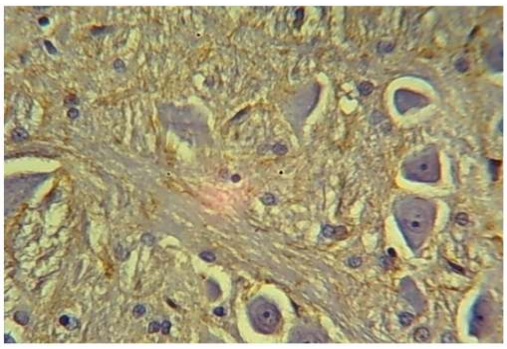

Fig. 6. Photomicrograph of cerebrum from experimental animal tested with *C. afer* extract, 200 mg/kg weight of rat

Shows positive expression of GFAP on Section of Rat's cerebrum. GFAP serves as a biomarker for brain damage (IHC. X400)

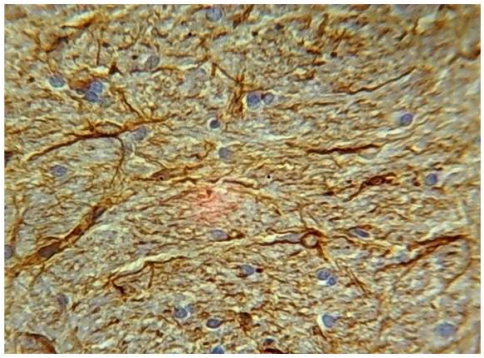

Fig. 7. Photomicrograph of cerebrum from experimental animal tested with *C. afer* extract, 500 mg /kg weight of rat

Shows positive expression of GFAP on Section of rat's Cerebrum. Indicated by increased astrocytic reaction (Astrogliosis). A biomarker for brain damage (IHC. X400.)

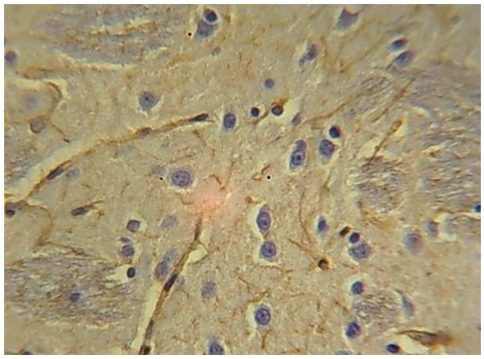

Fig. 8. Photomicrograph from cerebrum of experimental animal tested with *C. afer* stem juice, 5 ml /kgw of rat

Section shows positive expression of GFAP on Section of rat's cerebrum indicated by increased astrocytic reaction (IHC. X400)

The observed pathological changes in some of the cells of the cerebrum suggests that *C. afer* stem extract and juice may have some phytochemical constituents that are capable of altering the structure and hence the function of the CNS probably the alkaloids and the glycosides [25]. The bioactive compounds identified in the n-butanol fractions of *C. afer* leaves and stem was used to explain the folkloric use of the plant in the treatment of chronic inflammatory and oxidative stress related diseases [19]. The result of this study on the rat's model with ethanolic stem extract/juice suggests the need to be cautious in the use of the ethanolic fraction a as a herbal therapy. The observed vacuolations (V) in the cells present in the observed layers of the cerebrum (Figs. 3 and 4) indicate cell displacement suggesting some neuronal cell degeneration which could possibly be caused by the extract administration as it was not in the sections from control rats.

Glial Fibrillary Acidic Protein (GFAP) is the main intermediary filament of astrocytes the most abundant cell type in the human central nervous system (CNS) [26,27]. Astrocytes are supporting cells in the CNS and they react to insults or damage to the brain in an attempt to protect it. GFAP plays an essential role in maintaining shape and motility of astrocytic processes and contributes to white matter architecture, myelination and blood-brain barrier integrity. GFAP is a useful marker in traumatic brain injury, acute intracellular hemorrhage [28-30] and perhaps other neurological disorder [31]. Recent work has indicated that GFAP may serve as a serum marker of traumatic brain injury that is released after central nervous system cell damage [32]. The positive expression of the GFAP in the test groups including the group that received 5 ml/kg of juice suggests some levels of alteration in the normal molecular composition of the protein in the supporting cells (astrocyte) of the cerebral cortex. This indicates an anomaly and a disorder in the normal function of these cells as these molecules serve as biomarkers of brain injury. The release of GFAP from brain tissue into the blood stream may be caused by loss of astrocytic structural integrity due to necrosis and/or mechanical disruption and disintegration of the blood-brain barrier [29]. The upregulation of GFAP following different pathological events in the CNS is a process commonly known as reactive astrogliosis which was observed in this study from brain sections of test animals.

5. CONCLUSION

In conclusion, the result revealed that consumption of *Costus afer* ethanolic stem extract and juice. caused mild vacuolation of cells and reactive astrogliosis in the cerebrum of *Wistar* rats. These are indicative of a mild toxic effect on the cerebrum.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The principles of Laboratory animal care were followed. This study was approved by the ethic committee on the use of laboratory animal in the Department of Human Anatomy, Faculty of Basic Medical sciences of the University of Calabar, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ayyanar M, Ignacimuthu S. Plants as food and medicine: An ethnobotanical survey among Kanikaran Community in Southern India. Eur. J. of Nutrition & Food Safety. 2013;3(4):123-133.
2. Okwu DE. Phytochemical vitamins and mineral content of two Nigeria Medicinal plants. Int. J. of Mol. and Advance Science. 2005;1(4):375-381.
3. Enwuru BV, Ogbonnia SO, Nkemehule F. Evaluation of antibacterial activity and acute toxicity of hydriethanolic extract of *Stachytarpheta angustifolia* (mill) vahl. Afr. J. of Biotechno. 2005;7(11):1740-1744.
4. Iwu MM. Traditional Igbo Medicine Institute of Africa studies. Publication University of Nigeria, Nsukka, Applied Sciences. 2009; 3(4):21-25.
5. Anaga AO, Njoku CJ, Ekejiuba ES, Esiaka MN, Asuzu IU. Investigations of the methanolic leaf extract of *Costus afer* ker-Gawl for pharmacological activities *in vitro* and *in vivo* phytomedicine. 2004;11(2-3): 242-248.
6. Anyasor GN, Ogunwenwo KO, Oyelana A, Akpo Funwe BE. Phytochemical constituents and antioxidant activities of

aqueous and methanol stem extracts of costus *afer* Ker-Gawl (Crostaceae). Afri J. Biotechno. 2010;9(31):4880–4884.
7. Taiwo AO, Bolanle AA. The leaf essential oil of *Costus afer* ker-Gawl from Nigeria. Flavor Frag J. 2003;18:309.
8. Tchamgoue AD, Lauve RY, Tchokouaha PA, Tarkang JK, Gabriel AA. *Costus afer* possesses carbohydrate hydrolyzing enzymes inhibitory activity and antioxidant capacity *in vitro*. Evidence-Based Complem. and Alt. Medicine. 2015;2015:1-6.
9. ThankGod NK, Monago CC, Anaclatus FC. Activity of aqueous extract of *Costus afer* stem alone and in combination with metformin. Eur. J. of Biotehno. and Bioscience. 2014;1(5):19-25.
10. Momoh SY, Yusuf OW, Adamu MM, Agwu Coc, Atan FO. Evaluation of the phyto-chemical composition and hyperglycemic activity of methanolic leaf extract of *Costus afer* in albino rat. Brit. J. of Pharmacological Research. 2011;1(1):1–8.
11. Ijioma SN, Nwosu CO, Emenike, CU, Okafor AI, Nwankwo AA. Antinociceptive property of. *Costus afer* ker stem juice and ethanol leaf extract in albino rats. Comprehensive J. of Med. Sciences. 2014;2(2):14-19.
12. Anyasor, G N, Onajobi, FO, Odutola AO, Efere MO. Evaluation of *Costus afer* in vitro anti-inflammatory activity and its chemical constituents identified using gas Chromatography mass spectrometry analysis. J. of Costal Life Med. 2015;3(2):132-138.
13. Ituen EB, Udo UE. Phytochemical profile, Adsorptive and inhibitive behaviour of *Costus afer* on aluminium corrosion in hydrochloric acid. Der Chemical Sinica. 2012;3(6):1394-1405.
14. Omokhua GE. Medicinal and socio cultural importance of *Costus afer* (ker Grawl) in Nigeria. Afri. Res. Rev. 2012;5:282-287.
15. Ezejiofor AN, Orish CN, Orish EO. Effect of aqueous leaves extract of *Costus afer* ker Gawl (Zingiberaceae) on the liver and kidney of male albino wistar rat. Anc. Sci. Life. 2013;33(1):4-9.
16. Ayakeme, Tonkiri ES Essien, Akaniwor JO. Evaluation of the hepatoprotective and *in vivo* antioxidant activity of methanolic stem extract of Bush cane (*Costus afer*) in alcohol induced liver cirrhosis in Rats, in alcohol induced liver cirrhosis in rats. J. Biol and Food Sci Res. 2012;3(3):29-34.
17. Emeh CC, Abbey BW, Essien B. Hipolipidemic activity of aqueous extract of *Costus afer* stems in diet induced hyperlipidemic rats. Scholars Research Library. Archiv of Appl. Sci. Research. 2012;6(4):55-62.
18. Akpan MM, Odeomena CS, Nwachukwu CN, Danladi B. Antimicrobial assessment of ethanolic extract of *Costus afer* Leaves. Asian Journal of plant science and Research. 2012;2(3):335–341.
19. Anyasor GN, Onajobi FO, Osielesi O, Adebawo O. Proximate composition, mineral content and *in vitro* antioxidant activity of leaf and stem of *Costus afer* (Ginger lily) J. Intercultural Ethnopharmacol. 2014;3(3):128–134.
20. Ukpabi CF, Agbafor KW, Ndukwe O, Agwu A, Nwachukwu SN. Phytochemical components of *Costus afer* extracts and its alleviation of carbon tetrachloride induced hepatic oxidative stress and toxicity. Inter J. of Modern Botany. 2012;2(5):120-126.
21. Ukana DA, Uwemedimo EU. Aniekan EA. Evaluation of phytochemical proximate and mineral element composition ofstem of *Costus afer* (Bush Cane). Asian J. of Plant Science and research. 2012;2(5):617-621
22. Fischer AH, Jacobson KA, Rose J, Zeller R. Haematoxylin and eosin staining of tissue and cell sections. CHS PROTOC. Pdb. Prot 4968; 2008.
DOI: 10.1101/pdb.prot4986
PMID: 21356829(PUBMED)
23. Halliday GM, Gullen KM, Kril JJ, Harding AJ, Harasty J. Glial fibrillary acidic protein (GFAP) immunohistochemistry in human cortex: A quantitative study using different antisera. Neurosci Lett.1996;209(1):29-32.
24. Samuel CU, Chukwunonso K. The Acute and subchronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* Ker (Zingiberaceae). Mice. J. of Comp. Clin. Pathol. 2010;75-80.
25. Kennedy DO, Wightman EL. Herbal extracts of phytochemicals: Plant secondary metabolites and the enhancement of human brain function. Adv. in Nutritr. 2011;2:33-50.
26. Mayer CA, Brunkhorst R, Niessner M, Pfeilschifter W, Steinmetz H, Foerck C. Blood levels of Glial Fibrillary Acidic Protein (GFAP) in patients with neurological diseases. PLoS One. 2013;8(4):e62101.

27. Middeldorp J, Hol EM. GFAP in health and disease. Progress in Neurobiology. 2011;93(3):421-43.
28. Dvorak F, Haberer I, Sitzer M, Foerch C. Characterisation of the diagnostic window of serum glial fibrillary acidic protein for the differentiation of intracerebral haemorrhage and ischaemic stroke. Cerebrovasc Dis. 2009;27(1):37-41.
29. Brunkhorst R, Pfeilschifter W, Foerch C. Astroglial proteins as diagnostic markers of acute intracerebral hemorrhage-pathophysiological background and clinical findings. Translational Stroke Research. 2010;1(4):246-51.
30. Foerck C, Niessner M, Back T, Bauerle M, De Marchis GM, et al. Diagnostic accuracy of plasma glial fibrillary acidic protein for differentiating intracerebral hemorrhage and cerebral ischemia in patients with symptoms of acute stroke. Clin Chem. 2012;58(1):237-45.
31. Storon1 M, Petzol A, Plant GT. The use of serum glial fibrillary acidic protein measurements in the diagnosis of neuromyelitis optica spectrum optic neuritis. PLoS One. 2011;6(8):e23489.
32. Lumpkins KM, Bocchicho GV, Keled Jian K, Simad JM, MC Cunn M, Scalea T. Glial fibrillary acidic protein is highly correlated with injury. J. Trauma. 2008;4:778-782.

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