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Anti-Proliferative and Antioxidant Effects of Methanol Extract of the Root of *Conyza sumatrensis* Retz (Asteraceae)

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ABSTRACT

The anti-proliferation and antioxidant activities of *Conyza sumatrensis* was carried out using bench-top assay method. The anti-proliferation activities were carried out using methanol extract of the root of *Conyza sumatrensis* on guinea corn (*Sorghum bicolor)* radicle at 1-30 mg/ml. While the antioxidant activity was done using superoxide dismuthase (SOD), ferric reducing antioxidant power (FRAP) and α-diphenyl-β-picrylhydrazyl (DPPH) antioxidant test. In addition to the evaluation, the phytochemical constituents of the methanol extract of the root of *C. sumatrensis* was performed. The extraction of the plant material was done by cold maceration and concentration of the extract was done using rotary evaporator at 40^{o}C. Results from the study show significant growth inhibitory effect on guinea corn. An average growth length of 2.74 ± 0.20 mm, 2.93 ± 0.44 mm and 2.88 ± 0.40 mm were produced by the radicle of the control seeds of the methanol extract, aqueous fraction and chloroform fraction respectively after 24 hrs. While the seeds treated with 30mg/ml were 1.07 ± 0.44 mm, 1.28 ± 0.24 mm, 0.20 ± 0.12 mm for the methanol extract, aqueous fraction and chloroform fractions were 60.95%, 56.30% and 93.06% reduction in length respectively. After 96hrs, the control recorded an average length of 20.95 ± 4.69 mm, 34.45 ± 4.92 mm and 13.73 ± 2.86 mm in relation to 3.15 ± 1.15 mm, 11.38 ± 1.65 mm and 0.65 ± 20 mm produced by the seeds treated with 30mg/ml of the methanol extract, aqueous fraction and chloroform fraction respectively. This indicates reduction in growth by 88.18%, 66.97% and 95.27% respectively. The plant extract was shown to contain alkaloid, saponins, cardiac glycoside, tannins, flavonoids, steroids, terpenoids and anthraquinone. Also, the antioxidant property of the chloroform fraction, aqueous fraction and methanol extract were 4.86 ± 0.03mmol/min/mg, 2.75 ± 0.02 mmol/min/mg and 3.59 ± 0.64 mmol/ml/mg respectively for SOD test, 351.57 ± 22.03 mg CEQ/100gdw, 148.94 ± 43.47 mg CEQ/100gdw and 339.99 ±15.32 mg CEQ/100gdw respectively for DPPH test and 147.12 ± 71.07 g CEQ/100gdw, 35.16 ± 9.05 mg CEQ/100gdw and 22.28 ± 7.60 mg CEQ/100gdw respectively for FRAP test. Thus, indicating that the chloroform fraction has better antioxidant property than the aqueous and chloroform fractions. In conclusion, the result of this work supports the ethno-medicinal use of the plant in treating tumour-related ailments. However, further investigation using tumour cell line *in vitro* or *in vivo* may be necessary to confirm this claim.

INTRODUCTION

Global research into medicinal plants used in treating tumour-related ailments has become imperative due to the emergence of various forms of cancer diseases. *Conyza sumatrensis* is indicated in traditional herbal medicine as one of the plants used in treating tumour-related ailments. Traditional herbal medicines are getting significant attention in global health discussions. About 80% of the African population relies on herbal medicines to complement its health needs and many countries, including Nigeria, have been attempted by herbalists using medicinal plants widely distributed in the wild. In such communities, cure for life-threatening disease condition characterized by tumour production is attempted using medicinal plants. In the literature, there are a lot of compounds ranging from lignins like 5-methoxypodophyllotoxin (Van Uden W, Homan B *et al.*, 1992) to terpenoids like taxol (Prasain *et al.*, 2001) that were originally obtained from natural sources but were later developed to full anticancer agents. The World Health Organization defines traditional medicine as "the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness (WHO 2010). Practices known as traditional medicines include Ayurveda, Siddha medicine, Unani, ancient Iranian medicine, Irani, Islamic medicine, traditional Chinese medicine, traditional Korean medicine, acupuncture, Muti, Ifá, and traditional African medicine. Core disciplines which study traditional medicine include herbalism, ethnomedicine, ethnobotany, and medical anthropology. Majority of people living in developing countries rely on medicinal plants for their primary healthcare needs (Ekor 2013). Medicinal plants have important contributions in the health care system of local communities as the main source of medicine of majority of the rural population (Ahmed, *et al*, 2009). Out of the total 422,000 flowering plant reported from the world, more than 50,000 are used for medicinal purposes (Hamilton *et al.*, 2004). About 60% of the world population and 80% of the population of developing countries rely on traditional medicine. More than 4.5 billion people in the developing world rely on medicinal plants as components of their healthcare (Bhat *et al.*, 2013). The use of herbal remedies is more prevalent in patients with chronic diseases such as cancer, diabetes, asthma and end-stage renal disease. Multiple factors such as gender, age, ethnicity, education and social class are also shown to have association with prevalence of herbal remedies use. There are many forms in which herbs can be administered, the most common of which is in the form of a liquid that is drunk by the

patient—either an herbal tea or a (possibly diluted) plant extract (Sajwan. *et al.,* 2007). Whole herb consumption is also practiced either fresh, in dried form or as fresh juice. Several methods of standardization may be used to determine the number of herbs used. Different specimens of even the same plant species may vary in chemical content. For this reason, thin layer chromatography is sometimes used by growers to assess the content of their products before use. Another method is standardization on a signal chemical (Bashar & Said Omar *et al.,* 2011).

In living systems, oxidation is a basic part of the normal metabolic process, in which reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated (Vijayabaskaran *et al.,* 2010). Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can results in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging (Bimal *et al.,* 2011). Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body. There are plenty of antioxidant substances present in plants (fruits, vegetables, medicinal herbs, etc.) and the free radical scavenging molecules present in them are in the form of phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, tannins), nitrogen compounds (alkaloids, amines), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, (Govindarajan *et al.,* 2005). So, to maintain a healthy body, one should always increase the intake of foods rich in antioxidant compounds that lower the risk of chronic health problems associated with the above disease conditions (Bimal *et al.,* 2011). Naturally occurring antioxidants can be used in foods and also for prevention and treatment of free radical-related disorders (Middleton *et al.,* 2000) which can also be replaced by commercially available, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), which are quite unsafe to use and is restricted due to their carcinogenic effects (Vinay *et al.,* 2010). Nitric oxide (NO) is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signalling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities (Balakrishnan *et al.,* 2009). The most commonly used methods for measuring antioxidant activity are those which involve the generation of free radicals which are then neutralized by

antioxidant compounds. DPPH is a well-known radical and a trap ("scavenger") for other radicals (Solai *et al.,* 2010). Therefore, rate reduction of a chemical reaction upon addition of DPPH is used as an indicator of the radical nature of that reaction. Because of a strong absorption band centered at about 520 nm, the DPPH radical has a deep violet colour in solution, and it becomes colourless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm. DPPH method measures electron donating activity of other compounds in the mixture and hence provides an evaluation of antioxidant activity due to free radical scavenging. Any molecule that can donate an electron or hydrogen will react with DPPH, thus neutralizing its colour from a deep purple to a light yellow by electrons from the oxidant compounds. The concentration of DPPH at the end of a reaction will depend on the concentration and the structure of compound being scavenged (Balasundram *et al.,* 2018). The aim of this work is to evaluate the anti-proliferative and antioxidant activities of the methanol extract of the roots of *Conyza sumatrensis.*

MATERIALS AND METHODS

The fresh root of *C. sumatrensis* was harvested from the premises of Delta State University Abraka, Delta State Nigeria in December 2015. The plant was identified by Dr. E.O Ikepfan of the Department of Pharmacognosy, Delta State University Abraka, Delta State Nigeria.

Extraction of plant material

The fresh root was collected and washed clean with water. The roots were then air dried at room temperature. Thereafter, the roots were pulverized into powder and 1.5kg of the powdered roots was macerated using 7.5L of 70% methanol for 96 hours. The liquid extract was then filtered using a funnel plugged with cotton wool and the filtrate was concentrated to a semi-solid paste using a rotary evaporator maintained at 30^0C. The extract was weighed and stored in a refrigerator for further use.

Phytochemicals Screening

The qualitative screening was carried out in accordance with the standard protocol as described by Sofowora (2008). Also, the quantitative evaluation for flavonoids, saponins and alkaloids was based on earlier reported method by Edeoga *et al.,* 2005).

Thin layer chromatography (TLC) screening

Thin Layer Chromatographic Analysis (TLC): was carried out using Silica gel GF254 as stationary phase and three mobile phases [toluene: ethyl acetate (9.3: 0.7); acetone: water: toluene (5:2:3) and acetone: water: toluene (5:1:4). Visualisation was done using daylight, ultraviolet light (254 nm and 365 nm), and vanillin as spray reagents.

DETERMINATION OF THE GROWTH INHIBITORY EFFECT OF THE METHANOL EXTRACT, AQUEOUS AND CHLOROFORM FRACTIONS OF *C. SUMATRENSIS* ON GUINEA CORN (*SORGHUM BICOLOR)*

Growth inhibitory effect of the methanol extract of C. *sumatrensis*

About 3g of the crude extract was weighed, transferred into a 100ml beaker and dissolve with 30ml of distilled water. Thereafter, 21 petri dishes were prepared by fitting them with filter paper underlined with cotton wool 3 each for control, 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and 30 mg/ml and the petri dishes were labelled accordingly. Thereafter, 20 viable seeds were then placed in each of the petri dishes and 10ml of distilled water was then added to the control. Thereafter, the various concentrations were added to their respective petri dish and the petri dishes were incubated in a dark cupboard. The length of the radicle emerging from the seeds were then measured at 24, 48, 72 and 96 hours respectively.

Growth inhibitory effect of the aqueous fraction of *C. sumatrensis*

About 3g of the aqueous fraction was weighed, transferred into a 100ml beaker and dissolve with 30ml of distilled water. Thereafter, 21 petri dishes were prepared by fitting them with filter paper underlined with cotton wool 3 each for control, 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml, 20 mg/ml and 30 mg/ml and the petri dishes were labelled accordingly. Thereafter, 20 viable seeds were then placed in each of the petri dishes and 10ml of distilled water was then added to the control. Thereafter, the various concentrations were added to their respective petri dish and the petri dishes were incubated in a dark cupboard. The length of the radicle emerging from the seeds were then measured at 24, 48, 72 and 96 hours respectively.

Growth inhibitory effect of the chloroform fraction of *Conyza sumatrensis*

3g of the chloroform fraction was weighed, transferred into a 100ml beaker and dissolve with 30ml of distilled water. Thereafter, 21 petri dishes were prepared by fitting them with filter paper underlined with cotton wool 3 each for control, 1 mg/ml, 2 mg/ml, 5 mg/ml, 10 mg/ml,

20 mg/ml and 30 mg/ml and the petri dishes were labelled accordingly. Thereafter, 20 viable seeds were then placed in each of the petri dishes and 10ml of distilled water was then added to the control. Thereafter, the various concentrations were added to their respective petri dish and the petri dishes were incubated in a dark cupboard. The length of the radicle emerging from the seeds were then measured at 24, 48, 72 and 96 hours respectively.

Evaluation of the Antioxidant activity of the methanol extract, aqueous and chloroform fractions

About 1g of the crude extract was weighed and dissolved in 9ml of distilled water. 1.2g of the aqueous extract was also weighed and dissolved in 10.8ml of distilled water and 1.3g of the chloroform extract was weighed and dissolved in 11.7ml of chloroform.

Superoxide Dismutase (SOD)

About 0.2ml each of solution of the methanol extract, aqueous and chloroform fractions were measured using a syringe and transferred into separate test tubes respectively. Thereafter, 2.5ml of carbonate buffer was then measured and transferred into each of the test tubes, followed by the addition of 0.3ml of adrenaline and mixed properly by shaking the test tube. Thereafter, the UV-visible spectrometer was calibrated using distilled water and the absorbance of the three mixtures were then taken. A control solution was also prepared by mixing 5ml of distilled water with 2.5ml of carbonate buffer and 0.3ml of adrenaline, and its absorbance was also taken.

Ferric Reducing Antioxidant Power (FRAP)

The method of Benzie and strain (1999) was used. 5µl of the solution of the methanol extract was measured using a micro-pipette and transferred into a test tube, 3.995ml of FRAP reagent was added. Thereafter, the test tube was then placed in a lighted cupboard for 30min. The same procedure was carried out using the aqueous and the chloroform fractions respectively, a control was prepared using 5µl of distilled water containing 3.995ml of FRAP reagent. Thereafter, the absorbances of the various mixtures (crude, aqueous, chloroform and control) were taken in triplicate.

2-Diphenyl-1,1-Picryl hydrazyl (DPPH)

The method described by Elusiyan *et al.* (2011) was used. 5µl of the solution of methanol extract was measured and transferred into a test tube using a micro-pipette. 3.995ml of DPPH

reagent was added and allowed to stand for 30min. The procedure was repeated using the aqueous and chloroform fractions respectively. A control was prepared using water containing 3.995ml of DPPH reagent. Thereafter, the absorbance of the various mixture (crude, aqueous, chloroform and control) was taken in triplicate.

RESULT

Extract yield and Phytochemical Screening Test

It was observed that 1.5kg of the powdered plant material yielded 36.7g (2.45%) of the extract, the aqueous fraction yield 10.4g (34.66 %) and chloroform fractions yield 4.7g (15.67%) upon partitioning.

Table 1: Phytochemical screening of methanol extract of the roots of *Conyza sumatrensis*

Phytochemical Substance	
Alkaloid	+
Anthraquinone	+
Cardiac glycoside	+
Flavonoid	+
Phlobatannin	-
Saponin	+
Steroid	+
Tannin	+
Terpenoid	+

Legend:

+: positive

-: Negative

Table 1 shows the phytochemical constituents that were identified from the plant.

Growth Inhibitory Effect of the Methanol extract, Aqueous and Chloroform fractions of *C. sumatrensis* on the length of Guinea corn (*Sorghum bicolor)* radicle

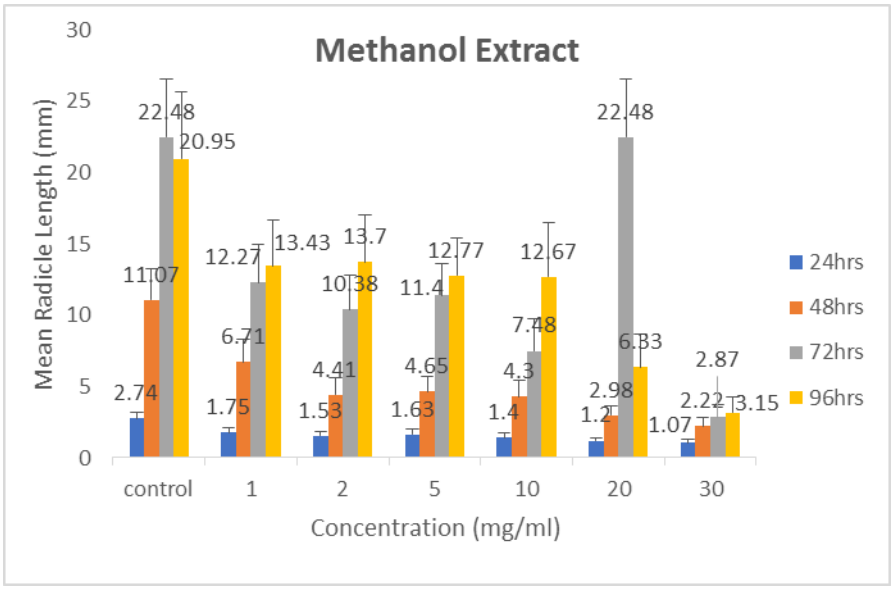

Figure 1: Growth inhibitory effect of methanol extract on guinea corn radicle length

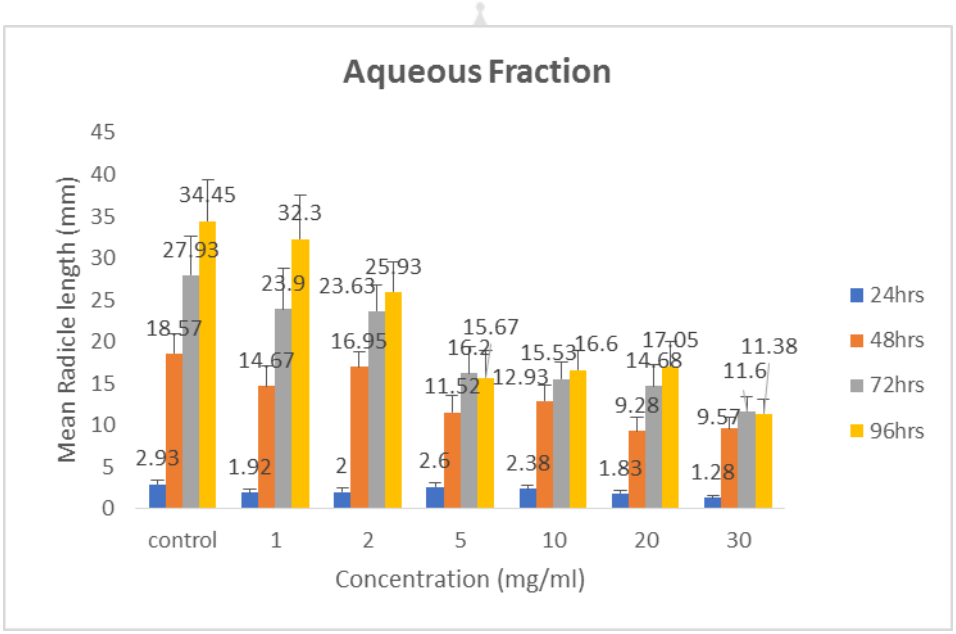

Figure 2: Growth inhibitory effect of the aqueous fraction on guinea corn radicle length

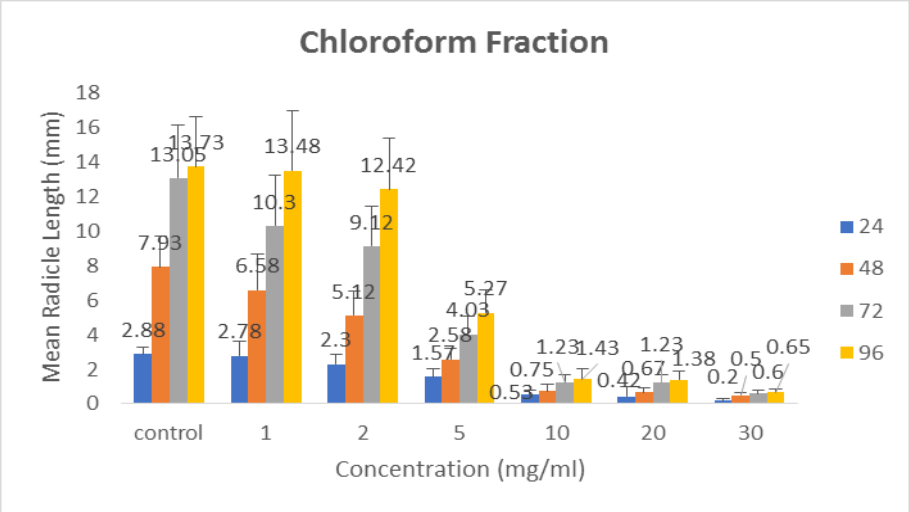

Figure 3: Growth inhibitory effect of chloroform fraction on guinea corn radicle length

Antioxidant activity of the methanol extract, aqueous and chloroform fractions of *C. sumatrensis*

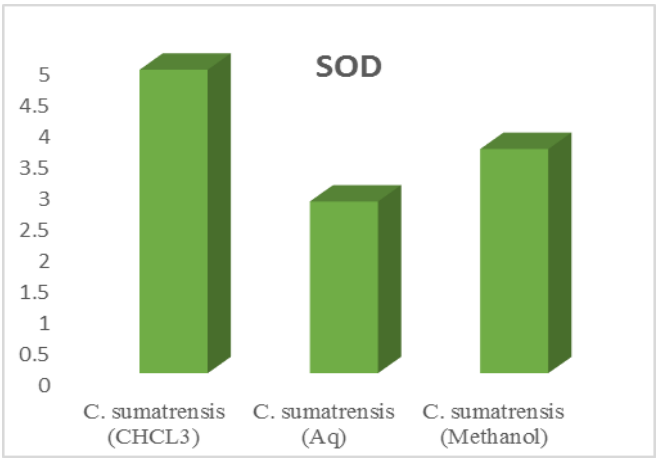

Figure 4: Antioxidant Activities of the Extract and fractions using SOD

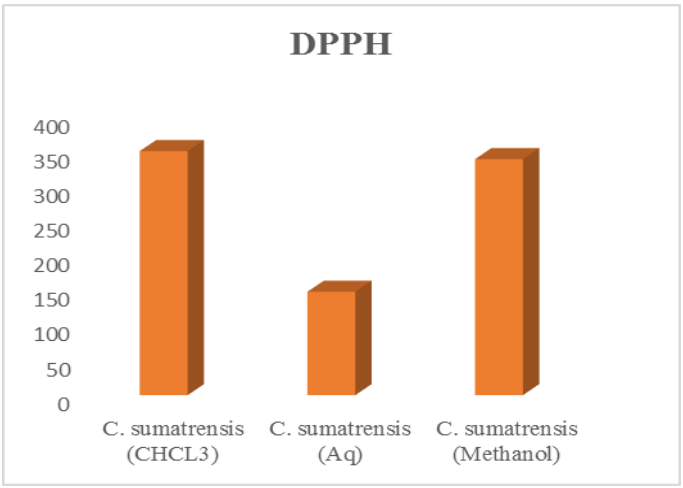

Figure 5: Antioxidant Activities of the Methanol Extract and Fractions using DPPH

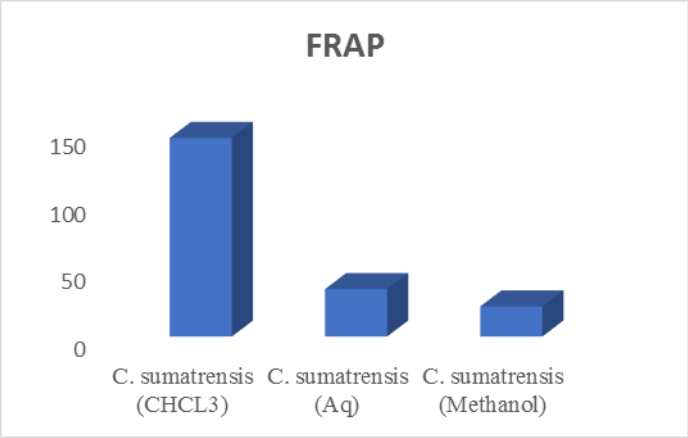

Figure 6: Antioxidant activities of the Methanol Extract and Fractions using FRAP

(a): growth on petri dishes of the control

(b) Picture of the petri dishes with 20m/ml of the methanol extract

(c) Picture of the petri dishes with 30mg/ml of the methanol extract

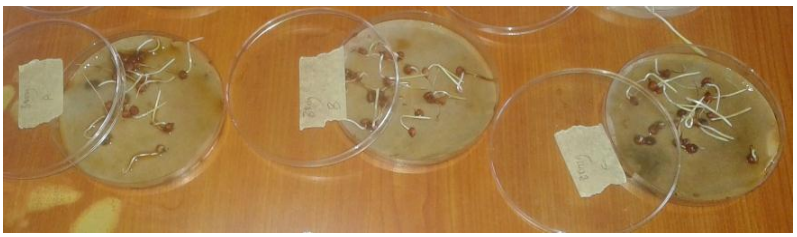

Plate 1: (a) control seeds of *S. bicolor*, (b) and (c) inhibitory effect of the root extract on the germination and growth of the seeds at 20mg/ml and 30mg/ml respectively.

DISCUSSION

Medicinal properties of plants are due to the presence of mixture of secondary metabolites which are stored in various specialized cells in the various tissues of plants and are usually extracted using various methods. Medicinal plant research currently will continue to be a useful resource in the search for new drugs (Atta-Ur-Rhaman, *et al.,* 2005). The revival of interest in the use of medicinal plant products for the treatment of various ailments is mainly due to increase awareness of the limited horizon of synthetic pharmaceutical products to control major diseases, high cost of currently available synthetic medicines, reported cases of adverse side-effects of modern medicines and perceived gentleness of natural medicines (Ayinde *et al.,* 2011). Cancer and tumour related ailments which are known to be among the leading causes of death are characterized by uncontrolled cell proliferation in the body, hence the need for research into medicinal plants with probable anti-proliferative effects in order to curb the high cost of treating the disease and the life-threatening side effects which usually accompany orthodox drugs. As earlier reported, research work in natural product chemistry must incorporate bioassays (Alcaraz *et al.,* 2004). Extracts must be screened for biological activity, the active extracts selected, fractionations directed with bioassays and the bioactive compounds identified and then exploited. The choice of guinea corn was based on the fact that meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions. Although seeds of other plants could have been used, *Sorghum bicolor* was preferred because of their relatively small size and their ability to give about 90 % germination rate within 24 hr. Preliminary phytochemical screening of the methanol extract of the plant was carried out to know the class of secondary metabolites it contained and the result showed the presence of saponins, tannins, flavonoids, cardiac glycosides and steroids as shown in (**Table 1**). Several phenolic compounds have been reportedly linked to have anti-proliferative antioxidant activities against three melanocytes cell lines (Ayinde, *et al.,* 2010). The methanol extract and fractions were observed to show a significant concentration dependent reduction in radicle length of the seeds as seen in (**Figure 1**). It was observed that as the incubation period increases, the length of the radicle continues to show a remarkable reduction when compared with the control. This may be due to the fact that components of the extracts may have interfered with certain biochemical processes directly or indirectly. Partitioning of the crude methanol extract into chloroform and aqueous phase further led to an increase in the growth inhibitory effects of both fractions. Aqueous and chloroform fractions like the crude methanol extract inhibited the guinea corn radicle length with

increased in concentration as shown in (**Figure 2 and Figure 3**), though the chloroform fraction was more effective than the aqueous fraction in this regard. The result of the aqueous and chloroform fraction revealed that the chloroform fraction inhibited the growth of radicle more than the aqueous fraction (**Figure 2**). Also, the aqueous and chloroform fractions were found to have antioxidant property like the methanol extract but the chloroform fraction was found to have higher antioxidant activity (**Figure 3**). This implies that the components responsible for the growth inhibitory effects and antioxidant activities are more present in the chloroform fraction than the aqueous fraction. Comparing the anti-proliferative activity with the antioxidant activity in (**Figure 4, 5 and 6**), it can be inferred that the antioxidant effect in relation to anti-proliferation may be due to the ability of the antioxidant constituent present in the *C. sumatrensis* to be able to mop off free radicles present in actively proliferative cells such as cancerous cells. Comparing the antioxidative and antiproliferative activities of *C. sumatrensis* with other plants like *Struchium sparganophora* it was observed that the latter is more potent than the former. Methanol extract of *S. sparganophora* was reportedly toxic on the tadpoles at 20 µg/ml while chloroform fraction exerted almost complete mortality at a concentration of 80 µg/ml. Furthermore, methanol extract remarkably reduced the growth of the guinea corn radicle at 4 mg/ml (Anaya *et al.*, 2018). Certain medicinal plants in the Asteraceae (Compositae) family have been reported to possess related activities. The allelopathic effects of *Ambrosia cumanensis* H.B.K. (Compositae) and the leaves and roots of *Piqueria trinervia* (Compositae) have been reported in the literature (De la Parra and Evans. *et al.*, 2002). Also, the antiproliferative effects of some Asteraceae (Compositae) species against human cancer cell lines have been observed (Saad *et al.*, 2009). Furthermore, the cytotoxic effects of *Sonchus oleraceus* and one of its constituents, loliolide, against mice, rat and human cell lines have been established. In the same way, the anticancer effects of the leaves of *Ageratum conyzoides L*. (Compositae) have been established scientifically (Adebayo *et al.*, 2010).

CONCLUSION

The results obtained has justified the ethnomedicinal uses of *C. sumatrensis* in treating tumour-related ailments by people from the western part of Nigeria. However, further research works are needed to fully justify this especially with the use of human cell lines. Also, there is need to establish the phytochemical constituents responsible for the anti-proliferative properties.

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