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Comparative phytochemical, cytotoxic and growth inhibitory effects of the leaf and root barks of *Sarcocephalus Latifolius* (J.E. Smith) E.A. Bruce (Rubiaceae)

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Abstract

Sarcocephalus latifolius is indicated in traditional herbal medicine as one of the plants used in treating tumor-related ailments in south western Nigeria. The cytotoxic and growth inhibitory effects of the methanol extract of the leaf and root bark were examined using the tadpoles of *Raniceps ranninus* and radicle length of *Sorghum bicolor* seeds respectively. The methanol extracts of the leaf produced 16.67 ± 2.38 % mortality at a concentration of 100 µg/ml and was eventually increased to 33.30 ± 7.60% mortality at 400 µg/ml while the root bark at concentrations of 200 and 400 µg/ml produced 100 % mortality over a period of 24 hr. An average growth length of 4.96 ± 0.77 mm was produced by the radicle of the control seeds in 24 hr and was reduced to 1.97 ± 0.68, 0.65 ± 0.47 and 0.27 ± 0.37 mm in the seeds treated with 10, 20 and 30 mg/ml of the leaf extracts. At 96 hr in which the control recorded a total length of 48.55 ± 6.21 mm in relation to 6.72 ± 2.13, 3.48 ± 0.64 and 2.38 ± 0.68 mm produced by the seeds treated with 10, 20 and 30 mg/ml of the leaf extract respectively which implies 86.16, 94.89 and 95.01 % reduction in growth length whereas there was 82.30, 95.60 and 98 .67 % reduction in radicle length by root bark extract at the same concentrations. The results implied the ethnomedicinal claimed of the plant particularly the root bark in the treatment of cancer.

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Introduction

All over the world, research into medicinal plants used in treating tumor-related ailments has become imperative due to the emergence of various forms of cancer diseases. In recent time, there has been an increasing amount of cancer research directed towards the investigation of plant-derived anticancer compounds, many of which have been used in traditional herbal treatments for centuries (Spiridon and Maria, 2001). With increasing recognition of herbal medicine as an alternative form of health care, screening of medicinal plants for biologically active compounds has become an important source of antibiotic prototypes and cancer-related drugs (Gordaliza, 2007; Meurer-Grimes *et al*, 1996; Rabe and Van Staden, 1997; Koduru *et al*., 2006). Therefore, in selecting crude plant extracts with potential useful properties, *in vitro* screening methods must be use for further in-depth chemical elucidation and pharmacological investigations (Meyer *et al*, 1998).

Treatment of cancer usually consists of various combinations of surgery, radiation therapy, and chemotherapy. Chemotherapy has remained the main stay in the treatment of various cancers. Despite the role of orthodox medicine as a source of anticancer drugs, the incidence of cancer is still on the increase. More so, their side effects are high and harmful to the patients. Hence, the need to focus attention on the use of medicinal plants (herbal medicine) as alternative source of anticancer agents for treating cancer. Natural and some synthetic compounds can prevent, suppress, or retard the progression of cancer. Natural products have played a significant role in the discovery and development of new anticancer agents and they represent a rich source of biologically active compounds (Abbas *et al*, 2010).

Locally known as Egbesi (Yoruba), uburu inu/nbitinu (Igbo) and marga (Hausa) in Nigerian languages, *Sarcocephalus latifolius* is one of the medicinal plants used in the treatment of cancer in parts of Ogun State in South-Western Nigeria (Soladoye *et al*, 2010).

Sarcocephalus latifolius (J.E. Smith) E.A. Bruce belongs to the family *Rubiaceae*. It is a shrub or small spreading tree that is a widely distributed in northern Cameroon and other African countries (Arbonnier, 2000). The medicinal uses of the plant includes the treatment of fever, pain, dental caries, septic mouth, malaria, dysentery, diarrhea and diseases of the central nervous system such as epilepsy (Arbonnier, 2000, Amos *et al*., 2005; Ngo Bum *et al*., 2009; Abbah *et al*., 2009). The aqueous extract of leaves of the plant has been used as a remedy for diabetes in northern Nigeria (Gidado *et al*., 2005). The anticonvulsant, anxiolytic and sedative properties of *S. latifolius* roots decoction (Ngo Bum *et al*., 2009) have already been reported as well as the antihypertensive and laxative activities (Akpanabiantu *et al*., 2005). This work was carried out for the first time to investigate the ethnomedicinal claims of the plant parts (leaves and root barks) in treating tumour related ailments, using two models ; growth length of guinea corn (*Sorghum bicolour)* seed radicles and the extent of their toxicity to the tadpoles of *Raniceps ranninus*.

Materials and methods

Collection and preparation of plant materials

The two morphological parts (leaves and root barks) of *Sarcocephalus latifolius* were collected in November 2012 from a play ground at Okhoro in Benin City. The identity of the plant was authenticated at Forest Research Institute of Nigeria (F.R.I.N.) Ibadan where a herbarium specimen FHI 109707 was deposited. The parts of the plant were spread on the laboratory table to dry for 3 days and were further dried in the oven maintained at 30°C (for the leaves) and 50°C (for the root barks). After this treatment, each material was ground to powder form using a laboratory electric milling machine (Chris Norris, England). Each powdered material were separately kept in an airtight container for subsequent use.

Extraction of plant material

500g of each of the plant materials (leaf and root bark of *Sarcocephalus latifolius*) were exhaustively

extracted by Soxhlet apparatus with absolute methanol at 70°C. The total methanol extract was concentrated to dryness under vacuum at 40°C using rotary evaporator (Eyela, Tokyo Rikikai Co. Ltd, Japan).

Phytochemical screening

Phytochemical screening of the methanol extract of the leaf and root bark of *Sarcocephalus latifolius* was carried out using standard methods described by Khandelwal (2006), Evans (2002) and Sofowara (1993).

Spectrophotometric quantification of phytochemicals

Quantification of flavonoids

The total flavonoid content was determined according to the aluminum chloride colorimetric method (Lin and Tang, 2007). 0.5 mL (100mg) plant extract was mixed with 0.5 mL of methanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCL_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK) and 2.8 mL of deionized water reagents. After incubation at room temperature for 40 min, the absorbance of the reaction mixture was measured at 415 nm on a UV-visible spectrophotometer (model UV-1601; Shimadzu®, Kyoto, Japan) and compared with the absorbance of deionized water as the control. Flavonoid contents were calculated on the basis of the calibration curve of Quercetin standard (2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one with 98% purity; Reg. 317313 Sigma, St. Louis, Missouri, USA). Data was expressed as milligrams Quercetin equivalents (QE) $100mg^{-1}$ plant extract.

Quantification of total phenolics

In order to measure the total phenolic content, Folin-Ciocalteau method described by Singleton and Rossi (1965) was used. 1 mL (100mg) of the extract fraction was mixed with 2.0 mL of 7.5% sodium carbonate (Na_2CO_3) and 2ml of Folin-Ciocalteau reagents. After incubation using water bath at 40°C for 45 min, the absorbance of the reaction mixture was measured at 765 nm on a UV-visible spectrophotometer (model UV-1601; Shimadzu®, Kyoto, Japan. Tannic acid was used as standard. The calculation of total phenol content was based on the calibration curve of the tannic acid standard and the data was expressed as microgram tannic acid equivalents (TAE) $100mg^{-1}$ plant extract.

Determination of cytotoxic effects using tadpoles (Raniceps ranninus)

Tadpoles scooped from ponds at Olomo Beach in Uhonmora village in Owan West Local Government of Edo State and were properly Identified in the Department of Animal and Environmental Biology, Faculty of Life Science, University of Benin. Ten tadpoles of similar sizes were selected with the aid of a broken Pasteur pipette into different beakers containing 30ml of the natural water from the habitat of tadpoles. This was made up to 49ml with distilled water. The mixture was made up to 50ml with 20, 40, 100, 200 and 400 µg/ml of the leaf extract in 5% DMSO (Obuotor and Onajobi 2000).

The assay was performed in triplicate and a control assay was performed using 50ml containing 1ml of 5% DMSO in distilled water. This procedure was repeated on the leaves and root barks.

Determination of antiproliferative effects on Sorghum bicolor (guinea corn) radicle length.

Seeds of *S.bicolor* (Guinea corn) were purchased from Uselu market in Benin City. A simple viability test was carried out by placing the seeds in distilled water. The viable seeds sank because of their denser embryonic tissues, unlike the non-viable seeds which floated and were therefore discarded. The floating seeds were decanted and separated from the viable ones. The viable seeds were washed with 95% ethanol for sterilization for 1 minute and were finally rinsed with distilled water.

Ten (10) ml different concentrations of the leaf methanol extract(1-30 mg/ml) containing 5% DMSO was poured into the petri-dish of about 9cm wide containing filter (Whatman No.1) underlay with cotton wool, after which twenty (20) of the sterilized seeds were spread on each of the petri-dishes. The

petri-dishes were incubated in a dark cupboard at room temperature and the lengths of the radicle emerging from the seeds were measured at 24, 48, 72 and 96 hours. The control seeds were treated with 10ml distilled water containing 5% DMSO (*Obuotor* and *Onajobi* 2000).The experiment was carried out in triplicates for all concentrations and controls while the radicle lengths were measured to the nearest millimetre. The procedure was carried out for both the leaves and root barks methanol extracts of *S. latifolius*

Statistical Analysis

All data were expressed as mean ± SEM and one way Analysis of Variance Anova statistical test using Graph pad Instant R version 2.05 (UK) was used to test for significance. P< 0.05 was considered Significant.

Results and discusion

Preliminary phytochemical examination of the methanol extract of the leaf and root bark of *Sarcocephalus latifolius* revealed the presence of alkaloids, tannins, saponins and flavanoids but devoid of steroid and anthracene derivatives (Table 1). Quantitative assay of flavonoids and total phenolics of the leaves and root bark indicated a higher amount in the root bark (Table 2).

Table 1. Result of the preliminary phytochemical screening of the methanol extract of the leaf and root bark of *Sarcocephalus latifolius.*

Phytochemical groups	Leaf	Root bark
Alkaloids	+	++
Anthraquinones	-	-
Cardiac glycosides	++	+++
Flavonoids	+	+++
Saponins	+	+++
Steroids	-	-
Tannins	+	++
Terpenes	+	++

+ + +: appreciable amount; + +: moderate amount; +: minute amounts;
-: not detected.

Effects of the methanol extract of the leaves and root barks of Sarcocephalus latifolius on tadpole mortality

The methanol extracts of the two morphological parts gave concentration dependent results. The methanol extracts of the leaf was observed to produce 16.67 ± 2.38 % mortality at a concentration of 100 µg/ml and was eventually increased to 33.30 ± 7.60% mortality at 400 µg/ml. However, at the maximum concentrations of 200 and 400 µg/ml, the methanol extract of the root barks produced 100 % mortality over a period of 24 hr (Fig. 1).

Growth inhibitory effects of the methanol extract of the leaves and root barks of sarcocephalus latifolius on radicle length.

As stated earlier for the cytotoxic effects, the methanol extracts exhibited a remarkable and significant concentration dependent reduction in the length of the radicle in all the treated seeds compared to the controls. An average growth length of 4.96 ± 0.77 mm was produced by the radicle of the control seeds in 24 hr and was reduced to 1.97 ± 0.68, 0.65 ± 0.47 and 0.27 ± 0.37 mm in the seeds treated with 10, 20 and 30 mg/ml of the leaf extracts. This inhibition was sustained till 96 hr in which the control recorded a total length of 48.55 ± 6.21 mm in relation to 6.72 ± 2.13, 3.48 ± 0.64 and 2.38 ± 0.68 mm produced by the seeds treated with 10, 20 and 30 mg/ml of the leaf

extract respectively (Fig.2). This reduction in growth length implies 86.16, 94.89 and 95.01 % inhibitions respectively compare to the control. These variations in length were observed to be significant at P< 0.01.

Table 2. Quantitative analysis of flavonoids and total phenolics of the leaves and root bark of *Sarcocephalus latifolius*.

Extracts	Phenolics(mg/100mg)	Flavonoids (mg/100mg)
Leaves	0.16 ± 0.03	0.24 ± 0.01
Root bark	0.36 ± 0.05	0.33 ± 0.01

Similarly, the controls to the seeds treated with the root bark had an average length of 3.4 ± 0.76 mm in 24 hr whereas the seeds treated with 10, 20 and 30 mg/ml of the root bark extract produced average lengths of 1.6 ± 0.45, 0.95 ± 0.24 and 0.45 ± 0.17 mm respectively. At 96 hr, the average length was observed to 54.97 ± 5.07 mm for the controls while the seeds treated with 10, 20 and 30 mg/ml of the root bark extract produced radicles whose average lengths were 9.73 ± 1.73, 3.42 ± 0.70 and 0.73 ± 0.21 mm respectively which implies 82.30, 95.60 and 98 .67 % reduction in radicle length compared to the controls (Fig.3). These variations were significant at P < 0.01.

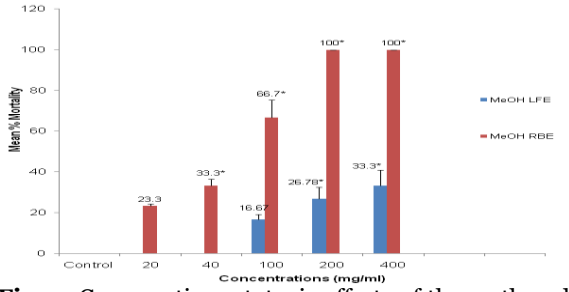


Fig. 1. Comparative cytotoxic effects of the methanol extract of the leaf and root bark of *Sarcocephalus latifolius* on tadpoles. Values are Mean ± S.E.M, n = 10. *Significant between Meth leaf and control. P<0.01. MeOH = Methanol, LFE= Leaf extract; RBE = Root bark extract.

Research work on medicinal plants with probable antitumour effects involve series of steps which may be fruitless if the material does not exhibit the claimed effects. In other to avoid wasting materials, certain bench top assay methods have been developed to predict the activities of medicinal plants in inhibiting proliferation of cells. Two of such methods employed in this work were the growth inhibitory effect of the extract on the radicles of guinea corn seeds and their lethal effects on the tadpoles of *Raniceps ranninus*.

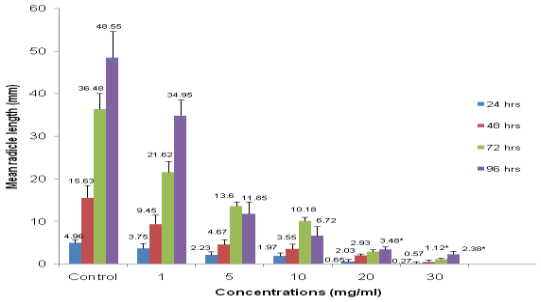


Fig. 2. The growth inhibitory effects of the methanol extract of the leaf of *Sarcocephalus latifolius* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20. *Significantly different from control P<0.05.

Bench-top assay methods have been variously used to investigate the ability of plant extracts to impart cytotoxicity on certain zoological organisms like the tadpoles, Artemisia. (Mc Laughlin, 1991). Apart from the reproducibility the methods provide, they are simple and can be used to screen medicinal plants with probable antitumor effects. The use of the organism mentioned above has been reported to be a measure of the plant extract to inhibit growth of tumor producing cells, induce dormancy in the plant seeds or produce allelopathic effects (McLaughlin *et al*, 1991).The tadpoles were used in this work due to their availability, particularly in the raining season. This of course could be a limiting factor in carrying out this kind of work in the drying season or in an environment where there is a dearth of water.

The anti-proliferative test carried out using the reduction in the length of the radicles suggested the

probable application of the plant material as herbicidal, alleopathic or antitumor agents. The plant extract exhibit this by interfering with the biochemical system and/or other growth related systems, like DNA division.

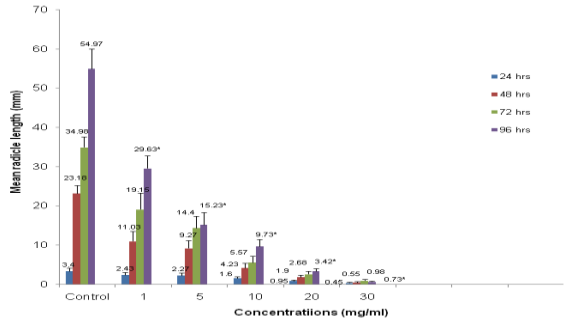
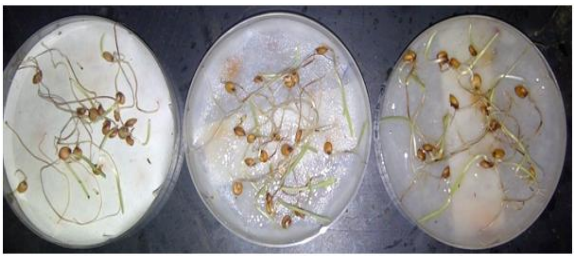


Fig. 3. The growth inhibitory effects of the methanol extract of the root bark of *Sarcocephalus latifolius* on the growth length of guinea corn radicle length. Values are Mean ± S.E.M, n = 20. *Significantly different from control P<0.01.

The phytochemical constituents found in the plant are: tannins, anthraquinone glycosides, saponins, alkaloids, flavonoids, cardiac glycosides and they vary in intensity. These suggested the probable presence of the major constituents responsible for these activities in the crude extracts of the root bark and also that their presence in a more concentrated form maybe responsible for the high percentage reduction of 98.67 % for the root bark extract showed over leaf extract.



(a) Control seeds.

(b) 20mg/ml of the leaf extract of *Sarcocephalus latifolius*.

(c) 30mg/ml of the leaf extract of *Sarcocephalus latifolius*.

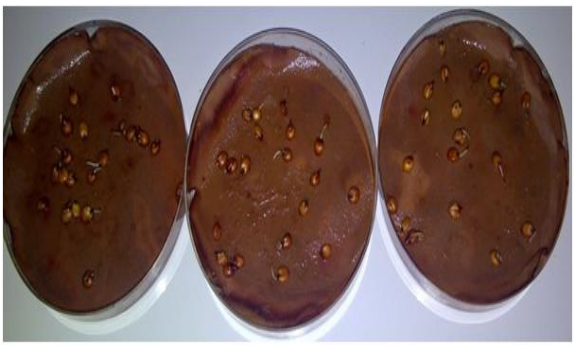
Plate 1. (a) Control seeds of *S. bicolor* (upper plates); (b) and (c) Inhibitory effects of the leaf extract on the germination and growth of the seeds at 20 and 30mg/ml.

Researchers have become interested in flavonoids and other phenolics for their medicinal properties, especially their potential role in the prevention of cancer and heart diseases (Kähkönen *et al*,1999). These compounds possess abroad spectrum of chemical and biological activities such as anti-allergic, anti-inflammatory, anti-microbial and anticancer and radical scavenging properties. This study revealed the total phenol contents of the leaves and root bark of *Sarcocephalus latifolius* expressed as microgram tannic acid equivalents of dry sample (standard plot: y =4.1491x, R^2=0.0491). The values were found between 0.16 to 0.36 µg tannic acid equivalent /g. The root barks were found to contains more phenolic compounds than the leaves. Similarly, using the standard plot of Quercetin equivalents (y = 4.2278x R^2 = 0.225), the root barks were also found to contain more flavonoids (0.33 µg) than the leaves (0.24 µg).

The use of guinea corn (*Sorghum bicolor*) was necessitated by the fact that meristematic tissues of seeds have the tendency to proliferate when exposed to favourable conditions and the extent of proliferation is reflected in the increase in the length of the radicles, produced in 96 hours in the control seeds. Although other seeds, such as maize (*Zea mays*) can be used, that of guinea corn was found to be more convenient because of its relatively small size. Also the availability of high and up to 90% can germinate within 24 hours.

(a) Control seeds.

(b) 20mg/ml of the root bark extract of *Sarcocephalus latifolius*.



(c) 30mg/ml of the root bark extract of *Sarcocephalus latifolius*.

Plate 2. (a) Control seeds of *S. bicolor* (upper plates); (b) and (c) Inhibitory effects of the root bark extract on the germination and growth of the seeds at 20 and 30mg/ml.

The present investigation revealed that the leaves and root bark of *S. latifolius* contain significant amount of phenols and flavonoids with the root bark containing more. The biological activity of the two morphological parts of the plant showed the root bark to be most active, hence from this study, it can be inferred that *S. latifolius* especially the root bark may likely have effects on tumour-producing cells. However, further chromatographic studies of the the crude methanol extract of the root bark may be required in other to isolate compounds responsible for these activities and further investigation using tumour cell lines *in vitro* or *in vivo* may be necessary to confirm this.

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