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The Evaluation of the Solvent Fractions of the Fruits, Leaves, and the Stem Bark of *Sarcocephalus latifolius* (SM.) E. A. Bruce for Cytotoxicity and Antioxidant Activities

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ABSTRACT

The geared trend in using medicinal plants in the management of various human ailments, cancer inclusive has gained significant consideration. Despite the innovations in technology, and the advent of foremost therapies, there is a growing inclination towards herbal medicine. In traditional medicine, *Sarcocephalus latifolius* (SL) is utilized to treat cancer; however, its efficacy in doing so has not been scientifically confirmed or supported by empirical data. The study evaluated the antioxidant and the cytotoxicity profile of the fruits, leaves and the stem bark of SL. These plant parts were harvested, washed, air-dried, pulverised and soaked in 100% methanol to obtain the crude methanol extracts (CMESL, MCESLL and CMESBSL). These were partitioned successively between n-hexane chloroform, ethyl acetate and methanol to obtain their respective fractions, N-hexane (HFSL, HFSLL and HFSBSL), chloroform (CFSL, CFSLL, and CFSBSL), ethyl acetate (EFSL, EFSLL, and EFSBSL), and the methanol (MFSL, MFSLL and MFSBSL). These extracts and fractions were subjected to *in vitro* antioxidant assays (DPPH, TAC, TPC and FRAP) and an *in vivo* cytotoxicity test using the brine shrimp lethality test (BSLT), to determine their LC_{50}. The results reveal that CFSL, CFSLL and CFSBSL is the most cytotoxic when compared to the other fractions, and for the antioxidant assays, the ethyl acetate fractions of the leaves and stem bark, EFSLL, and EFSBSL and the chloroform fraction, CFSL of the fruits, had the peak activity. *Sarcocephalus latifolius* plant demonstrates the potential as a rich reservoir for antioxidants and might have bioactive agents responsible for the anticancer effects.

Keywords: Medicinal plants, Sarcocephalus latifolius, antioxidant, cytotoxicity, bioactive agents, antioxidant

Introduction

The geared trend in using medicinal plants (MPs) in the management of various human ailments, cancer inclusive has gained significant consideration (Evbuomwan *et al*., 2023). In spite of the innovations in technology, and the advent of foremost therapies, there is a growing inclination toward herbal medicine in order to avoid the growing anxieties around the toxicities linked with orthodox managements (Evbuomwan *et al*., 2023). MPs include various types of plants used in herbalism, and some have medicinal activities (Mintah *et al*., 2022, Quinlan, 2022). The use of these plants (Herbs) for healing dates back to ancient times, highlighting a profound and enduring bond between humans and nature (Adil *et al*., 2024, Imah-Harry *et al*., 2025). These medicinal plants have become leading sources to harvesting novel bioactive agents and other ingredients used in novel drug development against various diseases (Obakiro *et al*., 2022, Shukla, 2023). Herbalism is defined as

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the use of plants or plant parts for either research or therapeutic reasons (Pale *et al*., 2022, Shukla, 2023). In Nigeria, for example, many people now manage a lot of diseases and ailments using natural plants or indigenous trees found around our environment (Mintah *et al*., 2022). The economic situation of the country presently has promoted the prompt switch from the conventional treatment of common ailments to the use of these herbal medicines.

In recent times, research has been channeled towards scientifically authenticating the claims of the healing efficacy of African herbs by old-style healers. This has become a major driver in scientific research since most of the circulated and unpublished printed ethno-medicinal data with valued and harmonizing information scattered in developing countries, depend on native preparations such as herbs, usually crude plant extracts, for their day-to-day needs (Lawal *et al*., 2010). An evaluation of the recognized adverse effects associated with their use in folklore medicine, has become essential and this is achieved by analyzing the plant's secondary metabolites (Ezenyi *et al*., 2020). Bioassays Lethality Test involving the use of Brine Shrimps is a valuable technique for evaluating cytotoxicity (Ikpefan, 2019). Plants metabolites are sometimes toxic to the larvae shrimps hence the investigation was carried out to ascertain the cytotoxicity profile of the solvent fractions of the aforementioned plant parts of *Sarcocephalus latifolius* (SM.) E. A. Bruce (Ikpefan, 2019).

Oxidative stress is a term used in defining a state of an imbalance between oxidants and antioxidants in a biological system, which could lead to the impairment of biomolecules in the body. It has been established that a high level of ROS in living beings causes several ailments including cancer and other inflammatory diseases (Al-Ragib *et al*., 2017). In a state of oxidative stress or in order to maintain general wellness of the body, antioxidants are used as dietary or medicinal supplements through various routes geared towards the suppression of damage caused by the presence of ROS in living beings (Al-Ragib *et al*., 2017). The attention of many scientists in recent times have been drawn to the reports of the use of antioxidants for deterrence and management of ailments and upkeep of general well-being. Africa (Nigeria inclusive) is blessed with a vast array of medicinal plants used both as preventive and curative alternatives in medical practice. This study is focused on one of these plants, namely *Sarcocephalus latifolius* (SL). *Sarcocephalus latifolius* (SL) is a perennial plant in sub-Saharan Africa utilized traditionally in the management and cure diseases like malaria fever, gonorrhea, wounds, cough, stomachaches, and problems related to the GIT and autonomic system. The fruits (Figure 1) of SL is utilized in traditional medicine to treat breast cancer; however its efficacy in doing so has not been scientifically confirmed or supported by empirical data.

Sarcocephalus latifolius, also called as *Nauclea latifolia* with a variety of common names like Pincushion tree or African peach in English and Scile maritime in French (Emmanuel *et al*., 2021), is a Sudano-Guinean species primarily found in west inter-tropical Africa. In Nigeria, it is referred to as "Egbesi" in Yoruba, "Uburu inu" / "Nbitinu" in Igbo, and "Marga" / "Tafashiya" / "Tuwon biri" in Hausa (Charles-Okhe *et al*., 2022, Obika *et al*., 2024), Figure 2. One unique thing about this plant is that the leaves remain green all through the year and flowers are seen anytime from January to mid-year. Indeed, traditional healers have frequently mentioned the use of *Sarcocephalus latifolius* extracts, sometimes with high citation frequency, while the use of *Sarcocephalus latifolius* is well documented in Southern Nigeria (Ezenyi *et al*., 2020). Moreover, *S. latifolius* is involved in the treatment of other diseases such as diabetes (Carlos *et al*., 2019, Sharma *et al*., 2020), AIDS, malaria (Ahoyo *et al*., 2019), menstrual disorders (Obika *et al*., 2024), intimate hygiene by African women in Kinshasa (DR Congo) (Ngbolua *et al*., 2014). Nigerian traditional medical practitioners treat diseases like fever, dysentery, hypertension and diabetes using roots and stem barks of SL (Figure 3), (Obika *et al*., 2024), while the leaf (Figure 2) is used treat diseases like cough or respiratory tracts infections (Lawal *et al.,* 2020), general fatigue, GIT disorders,

Figure 1, 2& 3: Matured Fruits, leaves and stem bark of *Sarcocephalus latifolius* (SM.) E. A. Bruce, Sighted On the Tree Just Before Harvesting at Iddo L.G.A. Oyo State, Nigeria.

body rashes and pimples (Filkpiere-Leonard, *et al*., 2023), malaria, and other fevers, according to research (Brooks *et al.,* 2020). Additionally, they treat piles, dysentery and renal diseases (nephroprotective), occasionally with the fruits (Filkpiere-Leonard, *et al*., 2023). Research establishes that the rich content of coumarins and polyphenolic compounds in the roots, which possess anti-inflammatory, antimicrobial, anticoagulant properties, explains the basis of its use in the management of diarrhoea infectious (Owolabi *et al*., 2020, Badiaga, 2021), and its ameliorative effects on Paracetamol-Induced liver damage (Da *et al*., 2023). There are also claims on the possession of antimalarial properties (Abbah *et al*., 2010) and hepatoprotective activities of the roots (Yesufu *et al*., 2014).

Sarcocephalus latifolius is thus a promising plant with these pharmacological and healing benefits, having been established effectual globally, especially in Africa in the management of several ailments in humans. However, there is dearth of information or empirical data to validate the claimed cytotoxic activity of *S. latifolius*, therefore, this examination on the cytotoxic, antioxidant and phytochemical profile of the solvent fractions of the of the aforementioned parts of the plant.

Materials and Methods

Chemicals and reagents

All reagents and organic solvents used for extraction and the vacuum liquid chromatography to get the solvent fractions (Sigma-Aldrich Chemical) were of a high analytical grade.

Plant sample preparation

The leaves, stem bark and fresh matured ripe fruits of *Sarcocephalus latifolius* (SM.) E. A. Bruce, were sourced along the forest paths along Eruwa, Iddo LGA of Oyo State, during the dry season, precisely November to December, 2023. While the leaves and stem bark were harvested from a particular shrub at once, the fruits were harvested one or two at a time. While the authenticity of the plant was confirmed, by Mr. Donatus Esimekhuain of Botany department, University of Ibadan, Nigeria, a voucher number (FHI 110092) and (UIH 23096), was allotted to the fruit, leaf and stem samples respectively. The samples were later deposited in the herbarium in the Pharmacognosy Department, University of Ibadan for future reference.

Processing of the sourced plant parts to the crude methanol extract and other solvent fractions.

The samples of (SL) were washed and air-dried at room temperature. Pulverization to powder was achieved using, using a mortar and pestle. Soaked in 100% methanol (Sigma, Aldrich Chemical Co. St. Louis, USA), (50g: 500ml) for 72 hours, cold maceration was completed. The filtrate was achieved with Whatman No.1 filter paper, to obtain the "methanol fraction filtrate" of the plant samples. The filtrate was concentrated using a Vacuum Rotary Evaporator (Stuart Rotavapor, UK) at 40ºC to obtain an extract, of a dark brown chocolate mass, dark green and black mass for the fruits, leaf and stem bark respectively. This was left at 37^0C for about four days, in a water bath to dry and be void of any solvent. The percentage yield was calculated and the crude methanol extracts (CMESL, MCESLL and CMESBSL) was freeze-dried using the lyophilizer and stored at 4°C. This resulted to the N− Hexane (HFSL, HFSLL and HFSBSL), chloroform (CFSL, CFSLL, and CFSBSL), ethyl acetate (EFSL, EFSLL, and EFSBSL), and the methanol (MFSL, MFSLL and MFSBSL) fractions using the VLC technique and the different solvents in accelerative order of polarity.

The percentage yield was 6.4% 14% and 10% respectively. The N-Hexane solvent fractions of the three plant parts was not used for the latter investigations.

Cytotoxic Analysis Using The Brine Shrimp Lethality Tests (Bslt)

Determination Of Cytotoxic Effects Of The Solvent Fractions Using Bslt

The brine shrimp lethality bioassay offers a simple, cost-effective, and low-material-consumption approach for preliminary biological screening (Krishnaraju *et al*., 2005). It acts as an initial filter, allowing for the identification of promising compounds before investing in more complex and expensive follow-up bioassays (Lall & Mayer, 1999). This method has been shown to be predictive of a substance's ability to be toxic to cells (cytotoxicity) and its effectiveness as a pesticide (Krishnaraju *et al*., 2005). Consequently, it has been widely adopted as a preliminary assessment for substances with potential cytotoxic and antitumor properties.

Hatching of Brine Shrimps (Artemia Salina)

This was done in two stages:

The preparation of the brine shrimp and the execution of the toxicity assay

First, brine shrimp (Artemia salina) were hatched from eggs in a controlled environment. This involved preparing a simulated sterile artificial seawater solution, also known as a brine solution. The solution was created by dissolving 38 grams of sea salt (sodium chloride) in 1000 mL of distilled water. The pH of this solution was then adjusted to 8.5 using 1N NaOH, and it was continuously aerated for 48 hours to facilitate hatching. Once hatched, the active brine shrimp were collected for use in the subsequent assay, following a methodology similar to that described by Krishnaraju *et al.* (2005).

Next, the toxicity assay was performed. Each test tube received 4.5 mL of the prepared brine solution. Appropriate dilutions of the test substance (extract) were made to achieve the desired concentrations. Then, 0.5 mL of the diluted test solution was added to each test tube. Subsequently, ten active brine shrimp were carefully transferred into each test tube using a glass capillary tube. After a 24-hour incubation period, the number of surviving shrimp larvae was counted. This data was then used to assess the lethality concentration, specifically the LD50 (Lethal Dose 50%), which represents the concentration of the test substance required to kill 50% of the exposed organisms.

Calculation

Calculation was expressed as a percentage of lethality of the brine shrimps for each concentration and control used.

For each tube, count the number of dead and number of live nauplii, and determine the % death. For this study the Clarkson's toxicity criteria were used for the toxicity assessment of plant extracts which was classified in the following order: extracts with LD_{50} above 1000 µg/ml are non-toxic, LD_{50} of 500 - 1000 μg/ml are low toxic, extracts with LD_{50} of 100 - 500 μg/ml are medium toxic, while extracts with LD50 of 0 - 100 μg/ml are highly toxic (Clarkson *et al*., 2004).

Determination of the antioxidant potentials

Determination of Total Phenol Content (Tpc)

Total phenolic compound contents were determined by the Folin-Ciocalteau method as described by (Ebrahimzaded *et al*., 2008a, b; Nabavi *et al*., 2008a). The extracts prepared from different parts of SL (CMESL, MCESLL, and CMESBSL) were tested by mixing 0.5 ml of each dilution with 5 ml of Folin–Ciocalteu reagent (previously diluted 1:10 with distilled water). After a 5-minute reaction period, 4 ml of 1 M aqueous Na_2CO_3 was added. The mixture was left to stand for 15 minutes, after which the phenolic content was measured colorimetrically at 765 nm. A standard curve was generated using Gallic acid solutions at concentrations of 0, 50, 100, 150, 200, and 250 mg/ml in a 50:50 methanol–water mixture. Total phenolic content was expressed as milligrams of Gallic acid equivalent per gram of dry sample, using Gallic acid as the reference standard.

Determination of 1,1-Diphenyl-2-Picryl Hydrazyl Radical (Dpph)

The antioxidant activity of the extracts (CMESL, MCESLL, and CMESBSL) was evaluated using the sTable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, following the methods described by Ebrahimzadeh *et al.* (2009a, b) and Ghasemi *et al.* (2009). Equal volumes of various concentrations of each extract were mixed with a methanolic DPPH solution (100 μM). After a 15-minute incubation at room temperature, the absorbance was measured at 517 nm. All measurements were performed in triplicate. Vitamin C, BHA, and quercetin served as standard reference antioxidants. The IC_{50} value represents the concentration of extract needed to neutralize 50% of the DPPH radicals.

Determination of Ferric Ion Reducing Antioxidant Power (Frap) Assay

The ferric-reducing ability of the samples was assessed based on the Oyaizu method, with minor modifications. In this procedure, the methanol extracts from the different plant parts of *Sarcocephalus latifolius* (CMESL, MCESLL, and CMESBSL) were prepared at concentrations ranging from 100 μl to 500 μl. Each sample was combined with 2.5 ml of 20 mM phosphate buffer and 2.5 ml of 1% (w/v) potassium ferricyanide, followed by incubation at 50 °C for 30 minutes. After incubation, 2.5 ml of 10% (w/v) trichloroacetic acid and 0.5 ml of 0.1% (w/v) ferric chloride were added, and the mixture was allowed to stand for 10 minutes. The absorbance was then recorded at 700 nm. Ascorbic acid served as the positive control. The modified aspect of the method was that all assays were performed in triplicate, with the results averaged to obtain the final values.

Determination of Total Antioxidant Content (Tac)

Total antioxidant capacity was determined using the phosphomolybdenum method as described by Prieto *et al.* (2005).

Preparation of Molybdate Reagent Solution

One millilitre each of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was mixed with 20 ml of distilled water. The mixture was then diluted with distilled water to a final volume of 50 ml.

Determination of Total Phenol Content

Hydro-alcoholic extracts from different parts of *S. latifolius* (CMESL, MCESLL, and CMESBSL) were prepared at concentrations ranging from 100 μl to 500 μl. Each volume was transferred into test tubes containing 900 μl to 500 μl of distilled water, respectively, followed by the addition of 500 μl of Folin–Ciocalteu reagent. After 5 minutes, 500 μl of sodium carbonate solution (100 mg/ml) was added. The mixtures were left to stand for 2 hours at room temperature, after which absorbance was measured at 765 nm. The phenol content of the extracts was expressed as Gallic acid equivalents (GAE). All measurements were performed in triplicate, and the mean values were calculated.

Statistical Analysis

Data were analyzed using SPSS Statistics (Standard Version 20; SPSS Inc., Chicago). Results from at least three independent experiments were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) was applied to determine statistical significance, with $p < 0.05$ considered statistically significant.

Results and Discussion

BSLT is a longtime speedy, universal laboratory procedure for bioactive compounds (Mclaughlin *et al.*, 1998). Bioactive compounds are nearly always virulent in elevated doses. A popular speech says, "Pharmacology is just pharmacological medicine at a lower dose, and pharmacological medicine is just pharmacology at the next dose". Therefore, *in vivo* morbidness in a very easy zoologic organism is often utilized as an expedient model to systematically observe and watch bioactive natural merchandise (Mclaughlin *et al.*, 1998). It also can be used to assess healthful plants for various medical specialty activities particularly toxicity tests as was the case during this study. Cytotoxicity primarily refers to the capacity of a compound to cause cellular death (Eisenbrand *et al.*, 2002); however, an equally critical mechanism involved is programmed cell death, which needs completely different strategies for its analysis and is of our interest during this study. The prevention of necrobiosis (programmed cell death) is additionally of great toxicological significance (especially in tumor-related endpoints). interestingly, *in vitro* toxicity assays are valuable tools for evaluating basic cytotoxic effects, particularly the inherent capacity of a substance to cause cell death through disruption of essential cellular functions. Such tests are important for determining appropriate concentration ranges for more detailed *in vitro* studies that generate meaningful data on outcomes such as genotoxicity, mutagenesis, and the induction of programmed cell death (Eisenbrand *et al.*, 2002). Determining the concentration that affects 50% of cells (TC_{50} or LC_{50}) also enables quantitative comparison of the responses of individual compounds across different systems, or multiple compounds within the same experimental system.The bioactivity of the extracts depends on what percentage of those shrimps can survive in the space of 24 hours of testing. It is thought of as one of the bench top procedures to be used in natural product chemistry (Mclaughlin *et al.*, 1998, Apu *et al.*, 2010). The results of the BSLT for the three plant parts are as in Table 1 below.

The ripe fruits of *Sarcocephalus latifolius* are traditionally used in the treatment of tumors; however, their mechanisms of action remain scientifically unverified. Empirical evidence to support these ethnomedicinal claims is limited. Additionally, the adverse effects associated with conventional cancer therapies highlight the need for safer, more selective, effective, and affordable alternatives. As a result, research interest has increasingly focused on natural bioactive compounds with the potential to selectively target tumor cells while sparing normal tissues. For example, foods rich in flavonoids, curcumin and quercetin have been shown to ameliorate incidence of several tumors or cancers (Wise *et al.*, 2011). Furthermore, a report from Pfeffer and Singh, (2018) have shown that people who consumed turmeric regularly in their diets, were reported to have the lowest incidence of most cancer types, clearly indicating the anticancer properties of curcumin, the bioactive compound in turmeric. Collectively, these properties of novel bioactive compounds present significant potential for innovative drug development (Ovadje *et al.*, 2015). In recent times, several medicinal plants have been exploited for their potency as antioxidants with very little or no adverse effects with profiTable feasibility.

In Table 1., MCESLL stands out as the least toxic

Table 1. Results of The Brine Shrimp Lethality Tests of the Crude Extracts and Solvent Fractions of the Fruits, Leaves, And Stem Bark of *S. latifolius*

FRUITS		LEAVES		STEM BARK	
Extract/ Fraction	**Lethal Concentration Lc50 (μg/ml)**	**Extract/ Fraction**	**Lethal Concentration Lc50 (μg/ml)**	**Extract/ Fraction**	**Lethal Concentration Lc50 (μg/ml)**
HFSL	31.18 ± 0.98^{b}	**HFSLL**	18.33 ± 0.04^{e}	**HFSBSL**	51.58 ± 0.06^{c}
CFSL	13.23 ± 0.16^{b}	**CFSLL**	13.23 ± 0.16^{e}	**CFSBSL**	61.43 ± 2.12^{d}
EFSL	15.41 ± 0.02^{b}	**EFSLL**	38.81 ± 0.01^{d}	**EFSBSL**	48.46 ± 0.03^{c}
MFSL	14.60 ± 1.23^{b}	**MFSLL**	34.37 ± 0.03^{d}	**MFSBSL**	58.13 ± 0.03^{c}
CMESL	11.86 ± 0.14^{a}	**CMESLL**	94.80 ± 0.03^{b}	**CMESBSL**	134.50 ± 0.04^{a}
CYCLO	61.82 ± 0.02^{b}	**CYCLO**	61.82 ± 0.02^{c}	**CYCLO**	51.58 ± 0.06^{c}

Keys

HFSL, HFSLL & HFSBSL = N-Hexane Fraction *Sarcocephalus latifolius* of the fruits, leaf and stem bark. CFSL, CFSLL & CFSBSL = Chloroform Fraction *Sarcocephalus latifolius* of the fruits, leaf and stem bark. EFSL, EFSLL & EFSBSL = Ethyl Acetate Fraction *Sarcocephalus latifolius* of the fruits, leaf and stem bark. MFSL, MFSLL & MFSBSL = Methanol Fraction *Sarcocephalus latifolius* of the fruits, leaf and stem bark. CMESL, CMESLL & CMESBSL = Methanol Crude Extract *Sarcocephalus latifolius* of the fruits, leaf and stem bark.

extract, with a significantly higher LC₅₀value (286.14 ± 10.09 µg/mL). MFSBSL and CYCLO (the standard) share a similar intermediate toxicity level, with LC₅₀values not significantly different from each other. EFSBSL and HFSBSL form another group with comparable intermediate toxicity. CFSBSL shows higher toxicity than EFSBSL and HFSBSL but lower than HFSL. HFSL is quite a toxic extract, with an LC₅₀value (31.18 ± 0.98 µg/mL). Interestingly, the N-Hexane fractions of all these plant parts are not used for the further assays of the study, because it is usually the oily extracts of the fractions. Additionally, the crude methanol extracts of all the plant parts stand as a reference that contains all the solvent fractions of the plant parts of *Sarcocephalus latifolius*. The three main solvent fractions the chloroform (CFSL, CFSLL & CFSBSL), Ethyl acetate (EFSL, EFSLL & EFSBSL) and the methanol fractions (MFSL, MFSLL & MFSBSL), were used for further studies in this investigation. Furthermore, of the three solvent fractions, CFSL and CFSLL is the most toxic with an LC_{50} of (13.23 ± 0.6µg/ml) respectively, when compared to the other fractions, following Clarkson's toxicity testing theory (Clarkson *et al.,* 2004). Therefore, the extracts of *Sarcocephalus latifolius* exhibit a wide range of toxicity levels against brine shrimp. The observed differences in LC₅₀likely result from variations in their chemical composition. Further research into the bioactive compounds responsible for the toxicity is warranted. It has also been documented that the curiosity in discovering antioxidants of natural origin in these plants has amplified, with the hope of substituting artificial antioxidants, which are usually poisonous and oncogenic with naturally occurring ones (Matsuura & Kurokawa, 2016). Antioxidants have the capability to defend organisms from harm triggered by free radical-induced oxidative stress and its ingestion may shift the equilibrium to an adequate antioxidant status (Patel *et al.,* 2010). Several investigations are ongoing universally (Yesufu *et al*., 2014), to discover natural antioxidants of plant origin with significant health benefits (Yesufu *et al*., 2014).

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay is commonly employed in plant biochemistry to assess the free-radical scavenging ability of plant-derived compounds (Baliyan *et al*., 2022). This method relies on spectrophotometric monitoring of changes in DPPH concentration following its reaction with antioxidant substances (Imran *et al*., 2022; Imran *et al*., 2025). Across the different parts of *S. latifolius* examined, radical-scavenging activity increased in a concentration-dependent manner. Among the solvent fractions from the leaves and stem bark, EFSLL and EFSBSL exhibited the strongest DPPH scavenging activity, indicating greater antioxidant potency relative to the other fractions, followed by the CFSBSL, then the MFSBSL and finally the CMESBSL (Figure 4a-c), showing the IC_{50} for DPPH radical-scavenging activity. It is documented that a higher total phenol content leads to a better DPPH-scavenging activity. The story was quite different for the fruits where the CFSL had the highest DPPH scavenging activity compared to the other solvent fractions. The results revealing that EFSLL and EFSBSL showed the best activity was in line with the results of Mohammad *et al*., 2023.

The Ferric Reducing Antioxidant Power (FRAP) assay was employed to evaluate the reducing ability of different solvent fractions of the various plant parts of *Sarcocephalus latifolius, used in this study.* This method measures the conversion of the ferric (Fe^{3+})–ligand complex to the intensely blue ferrous (Fe^{2+}) complex by antioxidant compounds under acidic conditions (Rubio *et al.,* 2016). The FRAP assay provides rapid, dose-dependent information

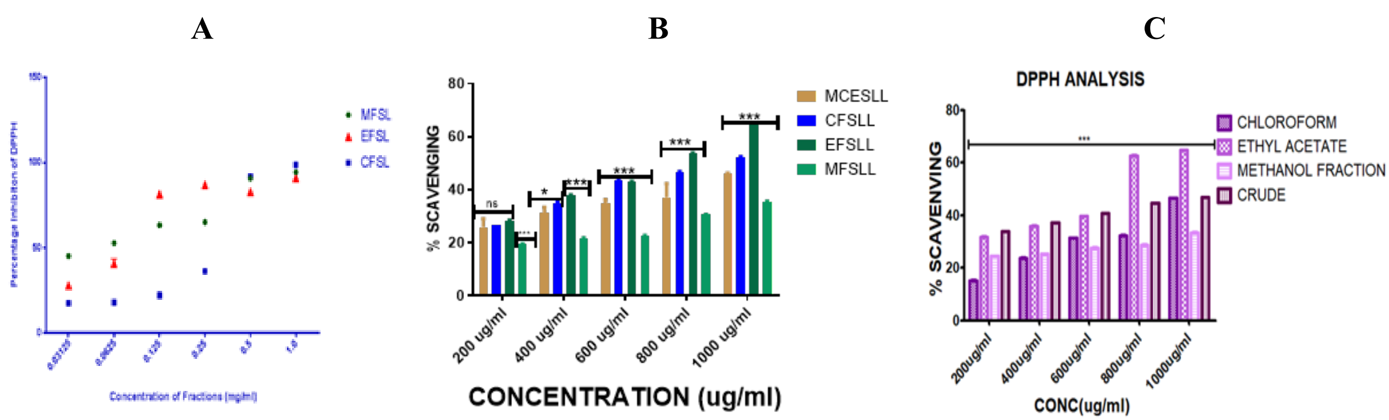

Figure 4a-c: The free radical scavenging activity of Fruit Fractions (CFSL, EFSL and MFSL), leaves (CFSLL, EFSLL and MFSLL) and stem bark (CFSBSL, EFSBSL and MFSBSL).

A) Each value is statistically significant at $p<0.05$, compared with control using the one-way ANOVA. B) values are not statistically significant at $p > 0.05$, * values are statistically significant at p< 0,05, *** means values are statistically significant at $p< 0.001$, compared with control using two-way ANOVA. C) ***means values are statistically significant at <p0.001, compared with control using two-way ANOVA

on the antioxidant capacity of individual samples, with greater color intensity indicating stronger reducing activity. The procedure is simple and highly reproducible. The reducing power of *S. latifolius* extracts was expressed as ascorbic acid equivalents (AAE) using a standard calibration curve ($y = 0.0016x – 0.2313$, $r^2 = 0.9421$) (Figure 5a–c). For the leaf and stem bark, it was noticed that EFSLL and EFSBSL had significantly higher Ferric Ion Reducing Antioxidant Power than the other solvent fractions.

This was followed by the CFSLL, CFSBSL> MFSLL, MFSBSL > MC ESLL, MCESBSL for the ethyl acetate, chloroform, and methanol fraction, respectively. This study is in line with the report of Rohan and Anup, 2013, who reported this same trend with the stem bark, root and fruits of the plant.

Total phenol compounds of *Sarcocephalus latifolius* was determined by Folin Ciocalteu method, are reported as Gallic acid equivalents by reference to a standard curve (y = 0.4967x - 0.586, r2 = 0.9593) (Figure 6a-c). The results for the leaves and stem bark reveal that EFSLL and EFSBSL had significantly higher TPC than the other solvent fractions. Phenolic compounds are abundant in plant-derived foods and are well recognized for their strong antioxidant properties. The elevated phenolic content observed in the EFSLL and EFSBSL fractions, as well as in the CFSL fruit fraction, likely accounts for their pronounced antioxidant activities, which is consistent with the findings reported by Ikepan *et al*. (2021).

B **C**

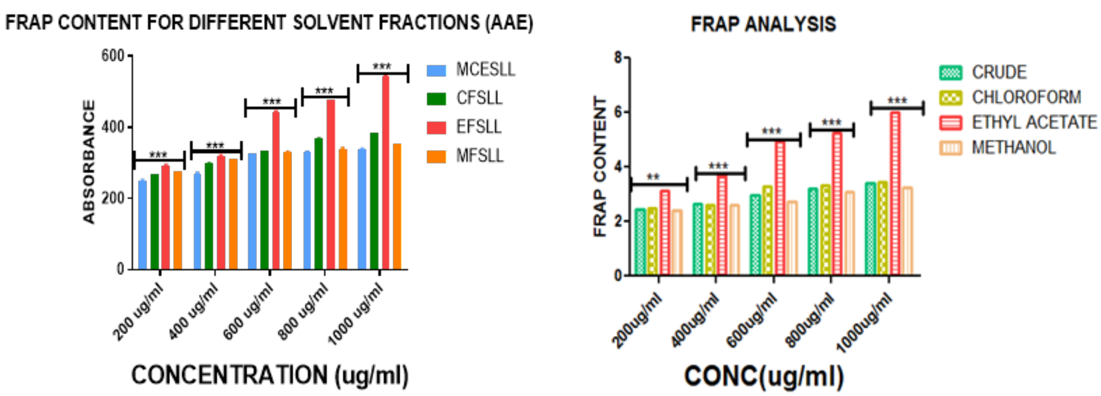

Figure 5b-c. The Ferric Ion Reducing Antioxidant Power (FRAP) leaves (CFSLL, EFSLL and MFSLL) and stem bark (CFSBSL, EFSBSL and MFSBSL). ** are statistically significant at $p < 0.01$, *** means values are statistically significant at $p < 0.001$, compared with control using two-way ANOVA.

A **B** **C**

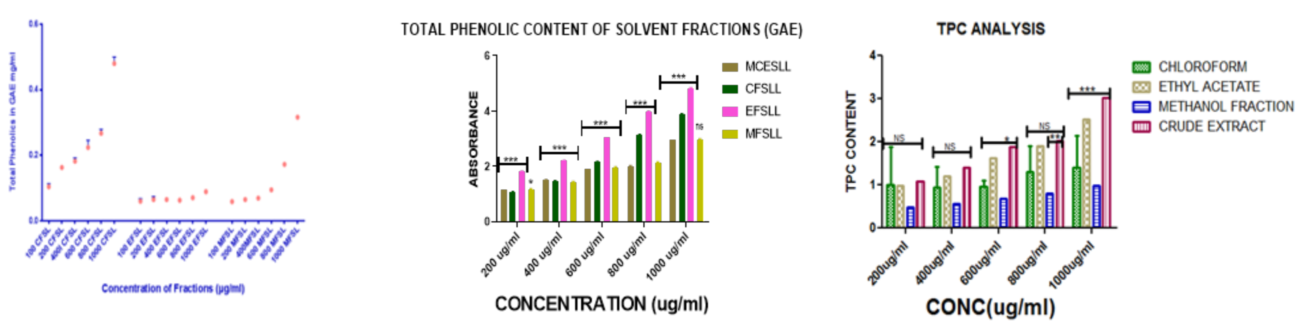

Figure 6a-c. Total phenol content of the three fractions, Fruit fractions (CFSL, EFSL and MFSL), leaves (CFSLL, EFSLL and MFSLL) and stem bark (CFSBSL, EFSBSL and MFSBSL). a) - Each value is statistically significant at $p<0.05$, compared with control using the one-way ANOVA. b) ns, values are not statistically significant at $p< 0.05$, * are statistically significant at $p< 0.05$, ** are statistically significant at $p< 0.001$, c) *** means values are statistically significant at $p< 0.01$, compared with control using two-way ANOVA.

As mentioned earlier in this discussion, the same trend is repeated here for the plants part. Total antioxidant capacity (TAC) is commonly used to assess the antioxidant status of biological samples and to evaluate the ability to counteract free radicals generated during specific diseases (Rubio *et al.,* 2016, Ally-Charles *et al*., 2024). Total Antioxidant Capacity (TAC) of *Sarcocephalus latifolius* was determined and reported as Ascorbic Acid Equivalence (AAE) by reference to standard curve ($y = 0.3785x$, $r2 = 0.8411$) (Figure 7a-c). EFSLL and EFSBSL revealed higher significant activity in Total Antioxidant Capacity than the other solvent fractions. In the fruits it is seen that the CFSL had the highest TAC activity compared to all other fractions. Therefore, the chloroform fractions of the three plant parts, fruits, leaves and the stem bark,
(CFSL, CFSLL and CFSBSL respectively, were the most cytotoxic when matched with the other solvent fractions and the ethyl acetate solvent fractions of the leaves and stem bark (EFSLL and EFSBSL) were observed to have the most powerful antioxidant potential, when matched the other solvent fractions, with the exception of the fruits where the chloroform fractions, CFSL topped the list.

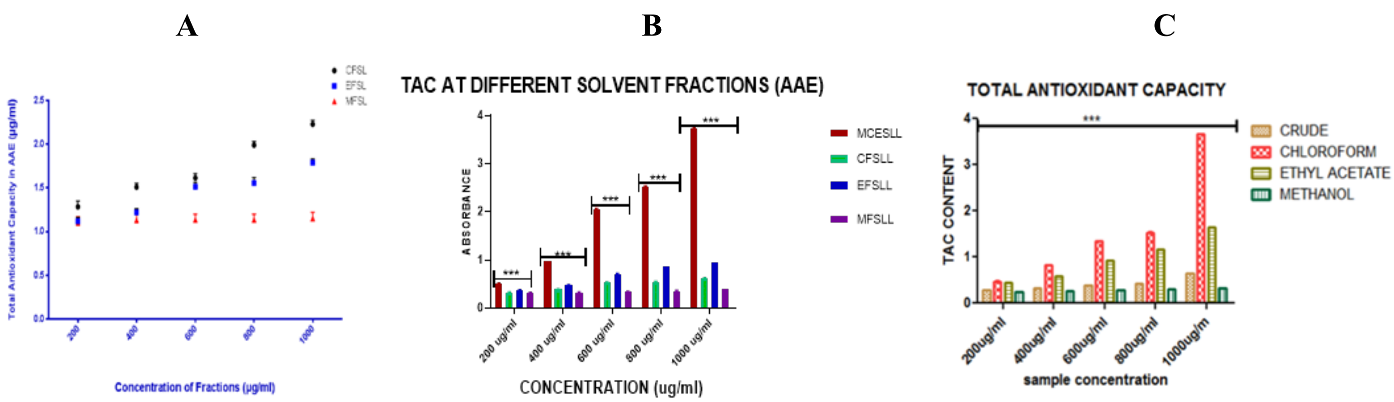

Figure 7a-c: Total Antioxidant Capacity (TAC) of the Fruit Fractions (CFSL, EFSL and MFSL), leaves (CFSLL, EFSLL and MFSLL) and stem bark (CFSBSL, EFSBSL and MFSBSL). a) At the highest concentration, CFSL had the highest TAC value of (2.23 AAEµg/ml) compared to the other fractions at the same concentration. - or - Each value is statistically significant at $p<0.05$, compared with control using the one-way ANOVA. (b-c) *** means values are statistically significant at $p< 0.001$, compared with control using two-way ANOVA.

Conclusion

The increasing prevalence of degenerative diseases and cancer, coupled with the numerous side effects linked to existing treatments such as chemotherapy, radiation, and surgery, is a major global concern. The need to look inwards and source the power of nature for an alternative cure or in synergy with the conventional therapy is on the increase globally. From this study, it could be said that every part of this plant has potential antitumor and anticancer properties and this explains why it is exploited in folkloric medicine in the treatment of cancer and tumors. Furthermore, the dual exhibition of a rich antioxidant strength, while exhibiting their anticancer properties, explains why it is used to manage so many common ailments in traditional medicines. *Sarcocephalus latifolius* (SL) plant demonstrates the potential as a good source of antioxidants and might have bioactive agents responsible for the anticancer effects.

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