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Anti-Salmonella and Radical Scavenging Activities of *Sarcocephalus latifolius* leaves and *Synclisia scabrida* roots against Human (*Salmonella typhimurium*) and Bovine Strains of *Salmonella enterica* species

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ABSTRACT

Typhoid fever is caused by *Salmonella enterica*, it can spread throughout the body affecting many organs. It could be fatal if not treated and can be transmitted through the ingestion of contaminated food or drink. *Sarcocephalus latifolius (*Sm.) E.A. Bruce (Rubiaceae) and *Synclisia scabrida* Meirs (Menispermaceae) are traditionally used in the management of typhoid by the people of Esan North East Local Government Area, Edo state. This study is to determine the anti-salmonella and radical scavenging activities of these selected plants. Powdered plant materials were extracted into methanol. The free radical scavenging activity and cytotoxicity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and brine shrimp lethality assay respectively. The antimicrobial activity of the extracts was assessed by the agar diffusion method and their Minimum Inhibitory Concentration (MIC) was determined against six strains of *Salmonella typhi*, from Cow (Co6b, Co11 and Co41), clinical strains (*Salmonella typhimurium* (sp), *Salmonella typhimurium* (SA) and a typed strain (*Salmonella typhi* ATCC 33458). Gentamycin was the standard drug used. The most active extract was subjected to liquid-liquid partitioning and the antimicrobial activity of the fractions determined. The DPPH results showed *Sarcocephalus latifolius* leaves extract (SLLE) having inhibitory concentration (IC_{50}) of 25.36 µg/mL while *Synclisia scabrida* roots extract (SSRE) had 14.04 µg/mL as compared with the standard drug (ascorbic acid) with IC_{50} of 9.26 µg/mL. The results of the brine shrimp lethality assay showed SLLE with LC_{50} of 735 µg/mL while SSRE had 158.67 µg/mL as compared to the standard drug cyclophosphamide with LC_{50} of 98.76 µg/mL. The MIC of the extracts SLL and SSR were 1.56 mg/mL and 0.78 mg/mL respectively. The result of the study justified the traditional claim of both plants in the treatment of typhoid and could provide lead for anti-typhoid fever drug development.

Keywords: Typhoid fever, *Sarcocephalus latifolius*, *Synclisia scabrida*

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INTRODUCTION

Salmonellosis remains one of the most frequent major public health concern. food-borne zoonosis, constituting a worldwide

Mortality rates from typhoid fever have been reported to be highest in Indonesia, Nigeria and India [1]. *Salmonella* infections are the second leading cause of bacterial food-borne illness in the world, the infection represents a considerable burden in both developing and developed countries [2-6]. The poor hygiene level of the live stocks in developing countries supports the introduction of pathogens to farm animals [7], while the main reservoir of non-typhoidal *Salmonella* in developed country is the intestinal tract of food-producing animals, this readily leads to contamination of foodstuffs and most human salmonella infections comes from the ingestion of such contaminated food of animal origin. The direct fecal-oral transmission from animal feces is also a possible source of human salmonellosis [8-11]. There is enough evidences to prove that foods from animal sources are the leading source of human salmonellosis when compared with person-to-person transmission [12-15]. Regulation of meat production in many countries is being enhanced but the problem of antibiotic resistance has become a global challenge. Multi-drug resistant strain of *Salmonella* spp. with reduced susceptibility to these drugs are often reported worldwide and the intestinal tract of food-producing animals is considered a reservoir for antibiotic resistance genes, posing a huge problem for humans for which cattle serves as a main source of food [16, 17]. Hence the continuous search for newer drug to militate against drug resistant salmonella in both human and animal.

Traditional medicine evolved from materials within the environment used by the people for survival. In Africa, traditional medicine was the only medical system available for health care before the advent of modern medicine, it is the oldest and the most assorted of all therapeutic systems. Even with the advent of orthodox medicine, traditional medicine is still the dominant medical system available to millions of people in Africa both in rural and urban communities [18-23]. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic and infectious diseases. Medicinal plants have been used for centuries as remedies because of their therapeutic values [24]. Presently, there is an alarming resistance to antibiotics by these microorganisms and the epidemic of multi-drug resistant typhoid fever is increasing by the day which could possibly lead to death [25-27]. Plants such as *Vernonia amygdalina* and *Carica papaya* have been reported for the treatment of salmonellosis in both animal and human infection [28]. It has been reported that antioxidants, plays a role in immunocompetence by increasing humoral antibody protection, resistance to bacterial infections [29], vitamin E, an important antioxidant is an example. Antioxidants are also known to induce biosynthesis of other antioxidants or defense enzymes [30]. In most infectious diseases, activation of phagocytes leads to the release of reactive oxygen species (ROS); oxidizing agents, that act in the processes that lead to the deactivation of viruses and the killing of bacteria. Many of the ROS are harmful to the host cells, antioxidant plays an additional role of protecting the host cells against the actions of ROS released by phagocytes [31-34].

Sarcocephalus latifolius Smith (Rubiaceae) is an evergreen, straggling, multi-stemmed shrub or a tree; it grows up to an altitude of 200 m. It is widespread in the humid tropical rainforest zone or in savannah woodlands of West and Central Africa. The three other related species *Sarcocephalus pobeguini*, *S. diderichii*, and *S. vanderguchtii* are forest trees. In folk medicine, the species *S. diderichii* and *S. orientalis* are used in the same way as *Sarcocephalus latifolius*. *Sarcocephalus latifolius* has an open canopy and terminal spherical head lined cymes of white flowers. The flowers are joined with their calyces and the fruit is syncarp. The tree flowers from April to June and the fruits are ripe from July to September. The wood of *Sarcocephalus latifolius* (Opepe wood) is termite resistant and is used as live stakes in farms. In West and South Africa, infusions and decoctions of the bark and leaves of

Sarcocephalus latifolius are used for the treatment of stomach pains, fever, diarrhoea, and against parasites, like nematodes in men and animals, and tropical diseases like malaria [35]. In Kano (Nigeria), *S. latifolius* is used as a chewing stick and as a remedy against stomach ache and tuberculosis [36]. In Ivory Coast, infusions and decoctions from stems and roots of *S. latifolius* are used against malaria by traditional healers [37]. In West and Central Africa, a co-generic species, *S. diderichii* is used for its insecticidal and anti-parasitic properties. In Gabon, Congo and Nigeria infusions of leaves and bark are employed against fevers [38]. In Kinshasa, DR Congo, extracts and preparations together with other plants are applied against diarrhea [39].

Synclisia scabrida (Meirs) of the family, Menispermaceae is a climbing shrub with slender stems that can be up to 40 metres long. These stems can climb high into the forest canopy, twining around other plants for support. It is a common shrub of tropical Africa present in southern Nigeria, Cameroon, Gabon, Democratic Republic of Congo and Angola [40]. It is commonly used as fodder for domestic animals but also has a folklore reputation as herbal remedy for lower abdominal pains, restlessness, mental strain and certain sexually transmitted diseases. Aqueous decoctions of the leaves, stem bark and root have been prescribed in ethno-medicine in cases of gastroenteritis. A leaf decoction is drunk to treat gastric ulcers. Pregnant women may tie a piece of liana around the waist to avoid spontaneous abortion [41].

In recent past, attention has been directed towards substantiating the claims of cure made by traditional healers thus providing scientific basis for their efficacy. We already reported an ethnobotanical survey carried out in Esan North East LGA, Uromi, Edo State for the treatment of typhoid and its complications [42], the aim of this work therefore, is to evaluate the antimicrobial activity of plants selected from ethnobotanical survey to assess their anti-*Salmonella typhi* activity.

MATERIALS AND METHODS

Plant collection

The leaves of *Sarcocephalus latifolius* and roots of *Synclisia scabrida* were collected in the Department of Environmental Biology, University of Ibadan and Ubierumu and Egbele villages of Esan North East LGA, Uromi, Edo State respectively. They were identified and authenticated in Forest Herbarium, Ibadan (FHI: 110364) and Ambrose Alli University herbarium, Ekpoma (voucher no: 555.) respectively. The plant materials were air-dried and milled into coarse powder.

Preparation and extraction of Plant Materials

Each plant materials were shade-dried at room temperature (27-33°C) and pulverized. The dried powdered materials were each macerated in methanol for 72 h with intermittent shaking (periodic agitation). The extracts were concentrated *in vacuo* at 45^{o}C using rotary evaporator. The extracts were further partitioned into *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol successively.

DPPH radical scavenging activity assay

In vitro Antioxidant Assay

The ability of the plant extracts to scavenge DPPH free radicals was assessed by the method described by Choudhary *et al* [43]. The 5 mg extracts and fractions were weighed on analytical balance and dissolved in methanol to obtain a stock concentration of 1000 g/mL and thereafter serial dilutions in the range of 200 - 6.25 µg/mL were made in a 96 well-plate. Diluted solutions (200 µL each) were mixed with 150 µL of methanol solution of DPPH in concentration of 0.04 µg/mL. After 30 min of incubation in darkness at room temperature (25 ˚C), the absorbance ($Absorbance_{sample}$) of the resulting solution was measured at 517 nm using the Ultra-violet spectrophotometer. Control simply contained all the reagents except the extract. Ascorbic acid was used as positive control while distilled

methanol as negative. All experiments were carried out in triplicate. The results were expressed as percentage inhibition.

$$\text{Inhibition (\%)} = \frac{\text{Absorbance}_{control} - \text{Absorbance}_{sample}}{\text{Absorbance control}} \times 100$$

Brine shrimp lethality assay

This test was done based on the method described by McLaughlin [44]. The brine shrimp eggs used were obtained from the Department of Pharmacognosy, University of Ibadan. The eggs were hatched in 3.8 g/L of natural seawater (from Bar beach, Ikoyi, Lagos). The larvae (nauplii) were placed in seawater 48 h to ensure survival and maturity before use. Stock concentration of plant extracts were made in not more than 5% methanol and diluted serially to obtain four concentrations. Each extract was dispensed in triplicates in clean test tubes of 10 mL volumes. To each tube was added 10 brine shrimps and incubated for 24 h at 29ºC after which each tube was examined for survival rate, and the surviving brine shrimps counted and recorded. The negative control consisted of 10 brine shrimps per tube in 5% methanol in seawater without plant extract while cyclophosphamide was used as the positive control. The 50% lethal concentration, LC_{50}, was computed using the Graphpad prism 6.0.

Antimicrobial Screening

Test salmonella strains

Local bacterial isolates from cases of gastrointestinal disorder, namely, *Salmonella* sp. from Cow (Co6b, Co11, Co41) and human (*Salmonella typhimurium* (SA)) were obtained from the Pharmaceutical Microbiology Laboratory, Department of Pharmaceutical Microbiology, University of Ibadan, Ibadan. The Department of Microbiology, Faculty of Sciences of the above University provided *Salmonella typhi* ATCC 33458, a typed strain. The Medical Microbiology Laboratory, University College Hospital, University of Ibadan, Ibadan provided the remaining *Salmonella* strain (*Salmonella typhimurium* sp.). All test *Salmonella* strains were screened using *Salmonella Shigella* Agar (SSA). The identity of each strain was confirmed by standard bacteriological methods by Cheesbrough [45].

Preparation of the culture media

The nutrient agar medium was prepared by suspending 28 g of the nutrient agar in 1 L of distilled water. The suspension was dissolved completely. It was then sterilized by autoclaving at 121ºC for 15 minutes.

Preparation of inoculums

Six strains of *Salmonella typhi* were used for this, three of the strains were from Cow (Co6b, Co11 and Co41) and three were from human (*Salmonella typhimurium* sp., *Salmonella typhimurium* (SA) and *Salmonella typhi* ATCC 33458). All microorganisms were isolated from clinical specimen except the typed strain (*Salmonella typhi* ATCC 33458). The test organisms were separately prepared by sub-culturing the pure isolates in nutrient broth and incubated at 37°C for 24 hours. Two loopful of the microbial culture were collected using sterilized (heat fixed) inoculating loop into 10 mL nutrient agar slant contained in sterilized universal bottles and then incubated at 37°C overnight for subsequent use. 0.1 mL of the overnight cultures of different organisms was then diluted with 9.9 mL nutrient broth to give 1 in 100 dilution equivalents which were then used for the study.

Sensitivity Test

Agar diffusion method was used in this assay. A weight of 8.4 g of nutrient agar was dissolved in 300 mL of water and was placed in a water bath at 100ºC for thirty minutes for homogenization. Thereafter, they were stabilized at 45ºC for 15 minutes. Each molten agar was inoculated with 0.2 mL of overnight broth culture of the test organisms. The seeded agar were poured into separate sterile petri dishes and allowed to set. Using a sterile cork borer (8 mm), six wells were bored in the set agar. The plates were labeled appropriately. The extracts were reconstituted by dissolving 0.5 g of each in 5 mL of methanol. The wells were filled with 100

mg/mL - 6.25 mg/mL of the methanol extracts from each of the plants. The remaining wells were filled with 10 µg/mL equivalent of standard drug (philogentamycin injection B.P. 80mg/2mL). The different methanol extracts were tested against six microorganisms *Salmonella* sp. from Cow (Co6b, Co11, Co41) and human (*Salmonella typhimurium* sp., *Salmonella typhimurium* (SA) and *Salmonella typhi* ATCC 33458). The plates were allowed to stand for one hour to allow adequate diffusion of the extracts and the drug. The plates were then incubated at 37ºC for 24 h. Zones of inhibition were measured in millimetre (mm) and the average found and recorded according to the method described by Mbata *et al* [46]. All experiments were done in triplicate.

Determination of Minimum Inhibitory Concentration

Nine concentrations of each extract were prepared. Thereafter, 2 mL of each concentration (100 – 0.39 mg/mL) of the extract was seeded into 18 mL of molten nutrient agar aseptically and the mixture was allowed to solidify. A 1 in 100 dilution of each test organism was then streaked on the plates using sterilized swab stick. Gentamycin (standard drug at 10 µg/mL) was introduced into the agar which served as the positive control. The lowest concentration preventing visible growth of the organisms was taken as the minimum inhibitory concentration of the extract.

RESULTS

DPPH radical scavenging activity assay

Analysis of the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the extracts in Fig. 1 shows that *Synclisia scabrida* root extract had an IC_{50} of 14.04 µg/mL which is comparable to the standard drug (ascorbic acid) with IC_{50} of 9.26 µg/mL while the *Sarcocephalus latifolius* leaves extract had IC_{50} of 25.36 µg/mL showing that SSLE had a higher scavenging activity.

Brine shrimp lethality assay

The results of the brine shrimp lethality assay showed LC_{50} of SSLE as 735 µg/mL while SSRE had 158.67 µg/mL as compared to the standard drug cyclophosphamide with LC_{50} of 98.76 µg/mL in Fig. 2.

Sensitivity Test

Analysis of the growth inhibitory activity as shown in Table 1 indicated that SSLE inhibited the growth of the six strains at higher concentrations with the highest inhibition at 100 mg/mL against Co11 (18.67.67 ± 0.54) as compared to the standard drug (gentamycin) with 18 ± 0.94 at 10µg/mL, it was mostly not active at lower concentration while slightly active against the typed strain (*Sal* ATCC 33458) which was only inhibited at 100 mg/mL with the value of 5.33±4.35.

Minimum Inhibitory Concentration

Synclisia scabrida roots extract (SSRE) had the best minimum inhibitory concentration (MIC) at 0.78 mg/mL as observed in Table 2. The ethyl acetate fraction SSL in Table 3 showed inhibitory activity on the test organisms, which were resistant to its crude extract. Impressive activity against the test organisms was observed with ethyl acetate fraction of SLL (Co11 22±0.00); *n*-butanol fraction (Co11 20±0.00 and *Sal* 20±0.00) and aqueous fraction (Co11 25±0.00) of SSR as seen in Tables 3 and 4 respectively. The MIC exhibited by the ethyl acetate fraction (3.13 mg/mL) of SLL against all organisms and aqueous fraction (3.13 mg/mL) of SSR against the Sal sp. and SA showed that the results were also remarkable as seen in Table 5.

DISCUSSION

The increasing resistance of microorganisms to already existing drugs is of major concern and necessary actions have to be made to combat this insurgence. Alternative source of medicine is now being sourced for with regards to the traditional way of treating these ailments. This study investigated the antimicrobial activity of selected plants from Southern Nigeria used traditionally in the treatment of typhoid. The brine shrimp lethality assay adopted in this study is used generally to evaluate

cytotoxicity effect and the presence of bioactive compounds in plant extracts [47]. Plants found to be cytotoxic may be a good candidate for cancer research. However, brine shrimp toxicity may not be extrapolated to mean toxicity to intact laboratory animals or man because the latter have more efficient mechanisms for breaking down similar substances found in foods of plant origin [47]. The decrease in absorption is used to measure the extent of radical scavenging. Free radical scavenging capacities of the extracts, measured by DPPH assay is shown in Figure 1. In comparison to the standard (ascorbic acid), SSR had a better radical scavenging activity than SSL. The result indicated that the extract was more active against the cow strain than the human strains. On the other hand, SSRE had a broad spectrum of activity, inhibiting the growth of all the six strains at all concentrations. The highest zone of inhibition of SSRE at 100mg/mL was observed as 43.33 ±1.67 mm in contrast to what Okoli and Iroegbu [47] reported that the ethanolic extracts of SSR produced inhibition zone of 24.5±1.50 mm against *Salmonella sp.*(*clin*). This could be deduced that methanol could be a better solvent that can be used to obtain the activities of the plant. Several researches have been carried out by various scientists on medicinal plants to treat salmonellosis, antimicrobial activity of *Cissus quadrangularis* and *Acacia polyacantha* in the treatment of salmonellosis in poultry and cattle gastrointestinal diseases from Benin was reported by Alain *et al* [48], they reported that the findings were as a result of the secondary metabolites present in the plants. Another report revealed that leaves of *Rytigynia canthioïdes, Securinega virosa, Dialium guineense, Pavetta corymbosa, Sansevieria liberica* and *Uvaria chamae* have *in-vitro* antimicrobial effect on a number of bacteria and fungi including *Salmonella typhimurium*[49]. SSRE in this study had the best minimum inhibitory concentration on (MIC) at 0.78 mg/mL. This is in contrast with the work reported by Okoli and Iroegbu [47] in which the ethanolic extract of SSR had an MIC of 6.25 mg/mL while SSLE had 1.56 mg/mL against Sal SA. This agrees with the study carried out by Oluremi *et al* [50] that the MIC of S. typhiUCH01 which they used was also at 1.563 mg/mL. The ethyl acetate fraction of SSL in this study showed inhibitory activity on the test organisms, which were resistant to its crude extract. This could be due to the fact that at the crude stage, the quantity of the components in the plants was either small or diluted and when fractionated, became concentrated and therefore exhibited activity on the organisms that were initially resistant to the crude extracts. The impressive activity exhibited by the ethyl acetate fraction of SLL; *n*-butanol fraction and aqueous fraction of SSR against the test organisms also indicated that the fractions were more active on the strain from the cow than the human strain which could mean that treating the disease from the infected animal with these plants could prevent it from being transmitted to humans. Bolou *et al* [51] observed in their study that *Terminalia glaucescens, Bersama abyssinica* sp. paullinioides and *Abrus precatorius* showed the most promising antibacterial properties inhibiting *Salmonella typhi.* The use of plants has become a vital part in the treatment of salmonellosis due to the alarming rate of antimicrobial resistance to already existing drugs. There is therefore the need to search for more medicinal plants that will be effective in the treatment of this disease.

It was observed that ethyl acetate fraction of *Sarcocephalus latifolius* leaves was more active than all other extracts asides the aqueous fraction of *Synclisia scabrida* root, and that the SLL fractions had the lowest MIC's as compared to that of SSR fractions, meanwhile at the crude stage, SSR was more active. These could infer that with further purification of SLLE more active compounds would be obtained. From this study therefore, the results justify the traditional claim of

extracts of *Synclisia scabrida* root and *Sarcocephalus latifolius* leaves in the treatment of typhoid and could be a lead to drug development for the treatment of typhoid fever.

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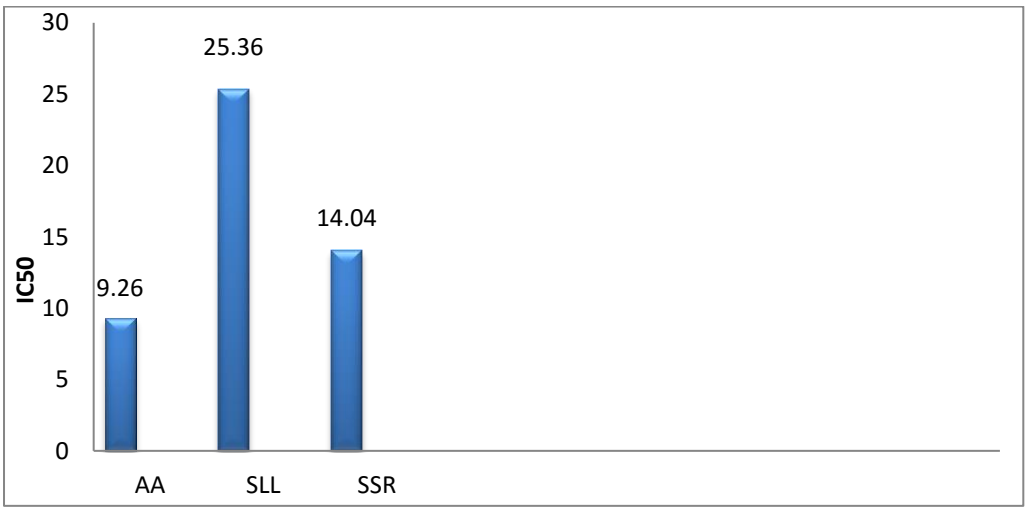
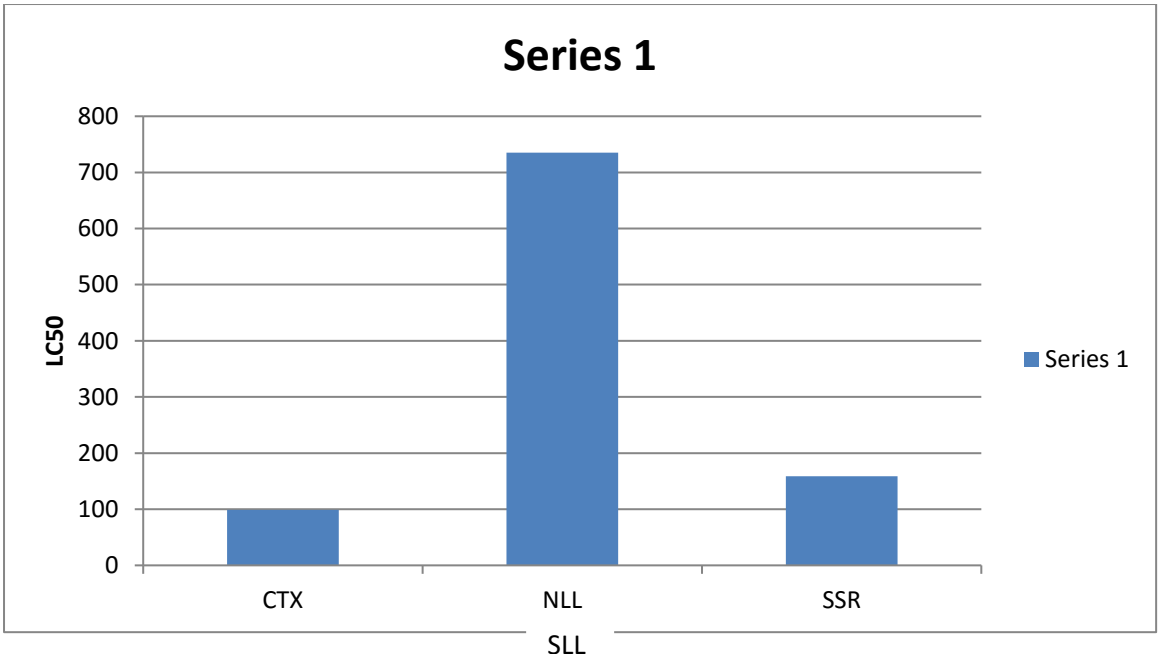


Key: AA- Ascorbic acid, SLL – *Sarcocephalus latifolius* leaves, SSR – *Synclisia scabrida* root

Figure 1: DPPH radical scavenging activity assay result



Key: CTX – Cyclophosphamide, SLL – *Sarcocephalus latifolius* leaves, SSR – *Synclisia scabrida* root

Figure 2: Brine shrimp lethality test result

Table 1: Standard deviation (S.D) and mean ± S.D of plant extracts on the various strains of *Salmonella typhi*

Conc. of extract (mg/mL)	Co6b	Co11	Co41	*Sal* ATCC 33458	*Sal* SP.	*Sal* SA
Sarcocephalus latifolius leaves						
100	16 ±1.89	18.67 ± 0.54	12.67± 0.54	5.33±4.35	14.67±0.54	14±0.00
50	9.33±0.54	11.33± 0.54	10.67± 0.54	-	4±3.27	12± 0.00
25	6 ±2.49	10±0.00	-	-	-	-
12.5	-	3.33±2.72	-	-	-	-
6.25	-	2.67±2.17	-	-	-	-
Synclisia scabrida roots						
100	43.33 ±1.67	20 ± 0.00	16± 0.94	16±0.00	19.33±0.54	16±0.00
50	18.33± 5.44	16.67± 0.54	14.67± 1.09	13.33± 0.54	17.33± 0.54	14.67± 0.54
25	15.33 ± 0.54	14±0.94	14±0.94	12.33±0.27	14± 0.00	11.33± 0.54
12.5	14 ± 0.94	12±0.00	11.33±0.54	10.67±0.54	12.33± 0.27	-
6.25	13.33 ± 0.54	10.67±0.54	11.33±1.09	3.33±2.72	12± 0.00	-
Gentamycin	18 ± 0.94	16±0.94	14.67±0.54	15.33±0.54	12±0.00	11.33±0.54

Key: - = not active;

Key: Co6b-Cow6b, Co11-Cow11, Co41-Cow41, Sal-*Salmonella typhi* ATCC 33458, Sal sp.= human strain. Sal SA= human strain

Table 2: Minimum Inhibitory Concentration (MIC) Results of the methanol extracts *Sarcocephalus latifolius* leaves and *Synclisia scabrida* roots

Test organisms	Concentration(mg/mL)	
	Sarcocephalus latifolius leaves	*Synclisia scabrida* roots
Co6B	100	1.56
Co11	6.25	1.56
Co41	100	1.56
Sal ATCC 33458	100	1.56
Sal SP.	25	1.56
Sal SA	1.56	0.78

Key: Co6b-Cow6b, Co11-Cow11, Co41-Cow41, Sal-*Salmonella typhi* ATCC 33458, Sal SP.= human strain. Sal SA= human strain

Table 3: Standard deviation (S.D) and mean ± S.D of the fractions of *Sarcocephaluslatifolius*leaves

Conc. of extract/ fraction (mg/mL)	Co6b	Co11	Co41	*Sal* ATCC 33458	*Sal* SP	*Sal* SA
n-hexane						
100	12 ±0.00	14 ± 0.00	12± 0.00	-	-	-
50	10± 0.00	12± 0.00	10± 0.00	-	-	-
25	-	10±0.00	-	-	-	-
12.5	-	-	-	-	-	-
6.25	-	-	-	-	-	-
Dichloromethane						
100	14 ±0.00	16 ± 0.00	12± 0.00	16± 0.00	12± 0.00	14± 0.00
50	12± 0.00	12± 0.00	10± 0.00	14± 0.00	10± 0.00	12± 0.00
25	10± 0.00	10±0.00	-	12± 0.00	-	-
12.5	-	-	-	10± 0.00	-	-
6.25	-	-	-	-	-	-
EtOAc						
100	16 ±0.00	22 ± 0.00	18± 0.00	16± 0.00	20± 0.00	16± 0.00
50	14± 0.00	18± 0.00	16± 0.00	14± 0.00	18± 0.00	14± 0.00
25	12± 0.00	16±0.00	14± 0.00	10± 0.00	14± 0.00	12± 0.00
12.5	-	14± 0.00	12± 0.00	-	10± 0.00	10± 0.00
6.25	-	13± 0.00	10± 0.00	-	-	-
n-butanol						
100	10 ±0.00	18 ± 0.00	10± 0.00	14± 0.00	14± 0.00	14± 0.00
50	-	14± 0.00	-	-	12± 0.00	12± 0.00
25	-	12±0.00	-	-	10± 0.00	10± 0.00
12.5	-	-	-	-	-	-
6.25	-	-	-	-	-	-
Aqueous						
100	-	16 ± 0.00	12± 0.00	16± 0.00	14± 0.00	14± 0.00
50	-	14± 0.00	10± 0.00	14± 0.00	12± 0.00	12± 0.00
25	-	12±0.00	-	10± 0.00	10± 0.00	10± 0.00
12.5	-	10± 0.00	-	-	-	-
6.25	-	-	-	-	-	-
Control (gentamycin)	14± 0.00	-	14± 0.00	18± 0.00	20± 0.00	16± 0.00

Key: - = not active;

Key: Co6b-Cow6b, Co11-Cow11, Co41-Cow41, Sal-*Salmonella typhi*ATCC 33458, Sal SP.= human strain. Sal SA= human strain

Table 4: Standard deviation (S.D) and mean ± S.D of the fractions of *Synclisiascabrida*root

Conc. of extrac/t **fraction** (mg/mL)	Co6b	Co11	Co41	*Sal* ATCC 33458	*Sal* SP.	*Sal* SA
***n*-hexane**						
100	12 ±0.00	12 ± 0.00	14± 0.00	16± 0.00	14± 0.00	12± 0.00
50	-	10± 0.00	12± 0.00	14± 0.00	12± 0.00	10± 0.00
25	-	10± 0.00	10± 0.00	12± 0.00	12± 0.00	10± 0.00
12.5	-	-	-	-	-	-
6.25	-	-	-	-	-	-
Dichloromethane						
100	-	16 ± 0.00	12± 0.00	-	-	-
50	-	14± 0.00	10± 0.00	-	-	-
25	-	12± 0.00	-	-	-	-
12.5	-	-	-	-	-	-
6.25	-	-	-	-	-	-
Ethylacetate						
100	18 ±0.00	14 ± 0.00	18± 0.00	18± 0.00	18± 0.00	12± 0.00
50	16± 0.00	12± 0.00	16± 0.00	16± 0.00	16± 0.00	10± 0.00
25	14± 0.00	12± 0.00	14± 0.00	14± 0.00	14± 0.00	-
12.5	12± 0.00	10± 0.00	12± 0.00	10± 0.00	10± 0.00	-
6.25	10± 0.00	-	-	-	-	-
nBuOH						
100	14 ±0.00	20 ± 0.00	18± 0.00	20± 0.00	18± 0.00	18± 0.00
50	12± 0.00	18± 0.00	16± 0.00	18± 0.00	16± 0.00	16± 0.00
25	10± 0.00	16± 0.00	14± 0.00	14± 0.00	14± 0.00	14± 0.00
12.5	-	14± 0.00	12± 0.00	12± 0.00	12± 0.00	10± 0.00
6.25	-	10± 0.00	10± 0.00	10± 0.00	-	-
Aqueous						
100	-	25 ± 0.00	16± 0.00	20± 0.00	20± 0.00	16± 0.00
50	-	20± 0.00	14± 0.00	18± 0.00	18± 0.00	14± 0.00
25	-	18± 0.00	12± 0.00	16± 0.00	16± 0.00	12± 0.00
12.5	-	16± 0.00	10± 0.00	14± 0.00	14± 0.00	-
6.25	-	14± 0.00	-	12± 0.00	10± 0.00	-
(gentamycin)	-	22± 0.00	-	20± 0.00	20± 0.00	20± 0.00

Key: - = not active;

Key: Co6b-Cow6b, Co11-Cow11, Co41-Cow41, Sal-*Salmonella typhi*ATCC 33458, Sal SP.= human strain. Sal SA= human strain

Table 5: Minimum Inhibitory concentration values for the partitioned fractions of *Sarcocephaluslatifolius*leaves

Test organisms	Minimum Inhibitory Concentration (mg/mL)									
	*Sarcocephaluslatifolius*leaves					*Synclisiascabrida* roots				
	Hexane	DCM	EtOAc	Butanol	Aqs	Hexane	DCM	EtOAc	Butanol	Aqs
S.entericaCo6B	*6.25*	6.25	3.13	25	-	-	25	25	6.25	6.25
S. entericaCo11	6.25	6.25	3.13	25	-	-	25	25	6.25	6.25
S. entericaCo41	6.25	6.25	3.13	25	-	-	25	25	6.25	6.25
Saltyphimurium ATCC 33458	6.25	6.25	3.13	25	-	-	25	25	6.25	6.25
Sal.typhimurium	6.25	6.25	3.13	12.5	6.25	-	25	25	6.25	3.13
Sal SA	6.25	6.25	3.13	12.5	6.25	-	25	25	6.25	3.13

Key: - = No inhibition,

Key: Co6b-Cow6b, Co11-Cow11, Co41-Cow41, Sal-*Salmonella typhi*ATCC 33458, Sal SP.= human strain. Sal SA= human strain

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