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Phytochemical and synergistic antimicrobial effects of *Morinda lucida, Anogeissus leiocarpus* and *Sarcocephalus latifolius* leaf extract

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Abstract: *Morinda lucida, Anogeissus leiocarpus and Sarcocephalus* latifolius are reputable medicinal plants with long history of uses in the treatment of variety of diseases. A combination of the leaves of *Morinda lucida, Anogeissus leiocarpus* and *Sarcocephalus latifolius* prepared as a decoction is used in Nigeria as remedy for *Lymphatic filariasis*, including complicated cases with wounds or sores. Extracts of the leaves of *Morinda lucida* (ML), *Anogeissus leiocarpus* (AL), *Sarcocephalus latifolius* (SL) and a combination of the three plants (mixed) were obtained by decoction. The extracts were subjected to phytochemical analysis using standard methods and high performance liquid chromatography. The extracts were also evaluated singly and in combination for DPPH antioxidant activity and antimicrobial effects against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella puemoniae, Bacillis subtillis, Candida albicans*. Phytochemical analysis showed the plants contained saponins, terpenes, sterols, phenolics and tannins. The HPLC spectrum of *Morinda lucida* showed thirteen peaks with betulinic acid, chlorogenic acid, caffeic acid, rutin and ferulic acid predominating. The HPLC spectrum of *Anogeissus leiocarpus* showed major peaks being gallic acid, chlorogenic acid, caffeic acid. The HPLC spectrum of *Sarcocephalus latifolius* showed major peaks being betulinic acid, gallic acid, caffeic acid, rutin. The HPLC spectrum of the combination showed major peaks as betulinic acid, gallic acid, chlorogenic acid, caffeic acid, rutin. *Anogeissus leiocarpus* extract had the highest antimicrobial and antioxidant activities followed by the combination. The study provided preliminary preclinical data supporting ethnopharmacological use of the combination therapy for treatment of *Lymphatic filariasis*.
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Keywords: *Lymphatic filariasis*, *Morinda lucida*, *Anogeissus leiocarpus*, *Sarcocephalus latifolius*, antimicrobial.

1. Introduction

A combination of the leaves of *Morinda lucida, Anogeissus leiocarpus and Sarcocephalus latifolius* prepared as a decoction is used in Nigeria as herbal remedy for treatment of *Lymphatic filariasis* (elephantiasis), including complicated cases with wounds or sores. *Morinda lucida* Benth belongs to the family Rubiaceae. It is called Brimestone tree (English), Erhan Ikpanro (Benin), Huka, Eze ogu (Igbo), Oruwo (Yoruba). It is an evergreen, small to medium sized tree up to 18-25m tall, with bole and branches often crooked or gnarled, bark smooth to roughly scaly grey to brown with some distinct purple layers. Leaves are opposite, simple and entire. The wood is yellow; the entire leaves are 7-12 cm long, petiole up to 1cm long, bladed elliptical, 6-18cm and 2-9cm, base rounded cuneate, apex, acute to acuminate, swing above, sometimes finely pubescene when young, latertufts of hairs in vein axils beneath and some hairs on the mid rib. *Morinda lucida* has long history of use and is safe for human consumption. Various extracts of the leaves have been reported to possess trypanocidal and antimalarial activities (Adenye and Agbaje, 2008; Adeleye *et al*., 2018). Inflorescence stalked head 4-7mm in diameter, 1-3 at the nodes opposite a single leaf, flowers are bisexual regular numerous

heterostylous, fragrant, calyx cup shaped, 2mm long, persistent, corolla salver-shaped, 1.5cm long, white or greenish yellow lobes ovate-lanceolate, up to 5mm x 2.5mm, ovary inferior 2-celled, style 8-11mm long with 2 stigma lobes 4-7mm long, stamens 5, inserted in the corolla throat with short filament. The fruit is drupe, severally together arranged into an almost globosely succulent syncarp 1-2.5cm in diameter, soft and black when mature, pyrene compressed ovoid, and up to 6.5 mm x 4 mm dark-red brown, very hard seeded, ellipsoid, 3.5 mm x 2 mm x 0.5 mm, yellowish soft. Flowering and fruiting occurs around October-January (Dhaarni *et al*., 2017).

Plants in the family Rubiaceae are used in management of many diseases including abdominal irritation, abortion, abscesses, anaemia, arthritis, ascardadis, ascite, asthemia, baby growth delay, chicken pox, conjunctivitis, constipation, cough, cryptococcal meaningitis, dermatitis, diabetes, diarrhea, dizziness, dysentery, dysmenorrheal, eczema, epilepsy, evil eye, evil spirit, fever, filariasis gastritis, general weakness, gonorrhea, headache, hemorrhage, hepatitis, hypertension, itchy, rashes, infant umbilical pains, internal inflammation, jaundice, kidney disease, measles, mycoses, obesity, oedema, ovarian cyst, paralysis and nerve diseases, pinworm, poisoning, pubic lice, respiratory infection, rheumatism, scabies, sexual impotence, snake bites, sterility, tapeworm, urinary tract infection; vomiting and wounds. Malaria and microbial infection are the main disease (Dhaarni *et al*., 2017).

Morinda lucida is used in cases of diabetes, hypertension, cerebral congestion, dysentery, stomach ache, ulcers, leprosy, and gonorrhea. It is mostly used in Nigeria for treatment of fever, in Cote d ivoire, a bark or leaf decoction is applied against jaundice and in DR Congo, it is combined as dressing of powdered root bark against itch and ring worm. The stem can be used in the treatment of piles (Dhaarni *et al*., 2017). *Morinda lucida* is used as stimulants, sedatives, arthritis, pulmonary troubles, rheumatism, vermifuges, laxatives, kidneys, diuretics, cutaneous and subcutaneous parasitic infection, veneral diseases, febrifuges, leprosy, yaws, tumors and cancers (Dhaarni *et al*., 2017).

Anogeissus leiocarpus (Guill and perr.) belong to the family combretaceae. It has the following vernacular names; English: African birch, Bambara, Anogeissus, chewstick; Hausa: Maarike, Marke; Yoruba: Ayin,Orin-odan, Pako ayin; Igbo: Abakaliki; Arabic: El-sahab; Nupe:Shici (Mezui *et al*., 2017). *Anogeissus leiocarpus* is graceful tree of Africa community known as Axle wood tree or African birch. It is a tall evergreen tree native to savannah of tropical Africa. It extends from the Sahel to forest zones and Senegal to Sudan and Ethiopia with Savannah regions as its habitats (Abdullahi *et al*., 2014). African birch is a slow growing evergreen shrub or small to medium sized tree, reaching up to 15-30mm in height. The bark is grey to mottled pale and dark brown, scaly, flaking off in rectangular patches, fibrous and exuding a dark gum. Leaves are alternate to nearly opposite, simple and entire, covered in dense silky hair when young. Flowers are pentamerous, pale yellow and fragment. Fruits are rounded samaras.4-10mm x 6-11mm x 2-2.5mm, with 2 wings and with a yellowish to reddish brown color and contain one seed.

Anogeissus leiocarpus is found in a large range of ecosystem from dry savannah to wet forest border. In wooded grassland and bush land, and in river banks in Ethiopia, Sudan, Cameroon, Congo Kinshasa, Benin, Cote d'ivoire, Ghana, Guinea, Mali, Niger, Nigeria and Senegal. In Sudanese traditional medicine, the decoction of the barks of *Anogeissus leiocarpus* is used against coughs. Rural populations of Nigerians use sticks for dental hygiene, the end of the sticks are chewed into fibrous brush which is rubbed against teeth and gum. Ivory Coast traditional practitioners use the plant for parasitic diseases such as malaria, trypanosomiasis, helminthiases and dysentery syndrome. In Togolese traditional medicine, it is used against fungal infections such as dermatitis and mycosis, leaves decoction is used against stomach infections (Victor, 2013). The plant is also used for the treatment of diabetics, ulcers, general body pain, blood cloths, asthma, coughing and tuberculosis (Victor, 2013). The genus *Anogeissus leiocarpus* belong to the family combretaceae widely distributed in Asia wild Africa. These plants parts (Stem, bark, leaf, seed, fruit and root of the plants are used as an ethnomedicine in Asia and Africa to treat various ailments like diabetes, fever, diarrhea, dysentery, tuberculosis, wound healing, skin disease (Eczema) snake and scorpion venom (Deeksha *et al*., 2016).

Many species of this family are used to treat inflammation, infectious diseases, bleeding malaria, diabetes and digestive orders (Gedson *et al*., 2012). African birch (*Anogeissus leiocarpus*) is widely distributed in Africa and its well known in African and Sudanese traditional medicine for treatment of many diseases such as toothache, diarrhea, laxatives, wound infections, sore feet, boils, cystphilotic, ulcers, skin diseases and infections. It shows strong antibacterial, antifungal and antimalaria activities (Bella and Jimoh, 2018).

Sarcocephalus latifolius (JE Smith) E.A Bruce belong to the family Rubiaceae. Vernacular names: English: African Peach, Pin cushion tree, Guinea peach, Bishops Head French: Scilleman tree, Medicinal squill, marine Hausa: Tafashiya, tashiyaiga, tafiyaigia, marga, Igala (Benue): UdeIgbo: Ubuluinu, Uvuru-iluYoruba: EgbesiI biobio: Mbom ibong

Itsekiri: ItuKilba: Molsa Cameroon (Etupin language): Koum louma. *Sarcocephalus latifolius* is a savannah tree or shrub up to 12m high, with a twisted bole up to 30cm in diameter, a spreadily open crown with a flexible entangled branches erect then dropping. The stem is cracked dark grey brown with fibrous reddish slash. It is multi stemmed and has an open canopy flowers with terminal spherical heads like cyma of small whitish flowers. The fruits is a syncarp, the individual fruits being fused together into a fleshy mass with characteristics pitted 1.17m surface (Mann *et al*., 2008). The seeds are minute and embedded in a pinkish flesh with straw berry scent, the tree flowers from April to June; Fruits ripens from July to September (Magili *et al*., 2014). It's a native of Africa and Asia, widely distributed throughout the forest and tropical forest of Benin, Burkina Faso, Cameroon, Democratic Republic of Congo, Ghana and Nigeria. *Sarcocephalus latifolius* can also be found in Senegal, and as far as Sudan, tropical and southern Africa. In Nigeria it is found in areas like Kontogora, Abuja, Akwa Ibom, Cross River, Enugu, Abakaliki and some other parts.

The genus *Sarcocephalus* is reputably used for treating malaria (Sourabie *et al*., 2013). Extracts (decoction of roots, bark, stem and leaves) of *Sarcocephalus latifolius* are use as anti-helminthic agent (Diarra *et al*., 2015). Plants from distributed savannah vegetation and usage by Bakongo tribes in Uige northern Angola. *Sarcocrphalus latifolius* have been similarly reported heavily in Nigeria for treating variety of disease such as Jaundice, yellow fever, measles. It was also cited among the most frequently used herbal medicine for the management of HIV-AIDS in Uganda (Kanteh and Noman, 2015).

The present study was aimed to evaluate the phytochemical profile and synergistic antimicrobial activity of *Morinda lucida, Anogeissus leiocarpus and Sarcocephalus latifolius* leaf extracts.

2. Materials and Methods

Plant material collection

Fresh leaves of *Morinda lucida* Benth, *Anogeissus leiocarpus* (D.C) Guill and Perr and *Sarcocephalus latifolius* J E Smith (E A Bruce*)* were collected from NIPRD garden and identified at NIPRD herbarium. The plant materials were identified and authenticated by expert taxonomist, Mr Akeem Lateefat the herbarium of the National Institute for Pharmaceutical Research and Development, Idu Industrial Area, P.M.B. 21 Garki, Abuja, Nigeria, where voucher specimen with number NIPRD/H/7038, NIPRD/H/7039 and NIPRD/H/7040 respectively where deposited. The plant materials were air-dried at room temperature for 2weeks, grounded to fine powder using electric grinder.

Preparation of plant extract

The air-dried leaves were chopped and weighed each at 300g for *Morinda lucida*, *Anogeissus leiocarpus*, and *Sarcocephalus latifolius*. Then the combination therapy of the three plants comprising 100 g each of *Morinda* lucida; *Anogeissus leiocarpus* and *Sarcocephalus latifolius* was also prepared as follows. The extracts were prepared by boiling with distilled water for 10-15 minutes using 750ml of water and allowed to stand overnight for 24 hours. The extracts were then filtered using a funnel blocked with cotton wool. The filtrates were dried over water bath to yield dark brown extracts. Percentage yields were 3.3%w/w, 4.9 %w/w and 4.8 %w/w respectively.

Phytochemical analysis

Phytochemical analysis was conducted on the extracts for secondary metabolites and using standard methods of Wakawa *et al*. (2018).

High performance liquid chromatography analysis

The bioactive constituents of extract was analysed by high performance liquid chromatography (HPLC) with UV diode array detector (UV-DAD). The HPLC consisted of Ultra-Fast LC-20AB equipped with SIL-20AC auto-sampler; DGU-20A3 degasser; SPD-M20A UV-diode array detector; column oven CTO-20AC, system controller CBM-20Alite and Windows LC solution software (Shimadzu Corporation, Kyoto Japan); column, 5µm VP-ODS C_{18} and dimensions (4.6 x 150 mm). The chromatographic conditions included mobile phase: 0.2% v/v formic acid and acetonitrile (20:80); mode: isocratic; flow rate 0.6 ml/min; injection volume 10µl of 100 mg/ml solution of extract in water; detection UV 254 nm. The HPLC operating conditions were programmed to give solvent B: 20%. Column oven temperature was 40 ^{o}C. The total run time was 30 minutes. Flavonoids and phenolic acid standards such as apigenin, rutin, quercetin, caffeic acid, ferulic acid were employed for the identification of the phytoconstituents by comparing the retention time under similar experimental conditions (Lamorde *et al*., 2016).

Antimicrobial and antioxidant evaluations.

Test Organisms

Pure clinical isolates of Bacillus subtilis, Klebsiella pneumoniae, collected and biochemically confirmed from Diagnostic Laboratory of NIPRD clinic and American Typed cultures of Escherichia coli (ATCC25952), Staphylococcus aureus (ATCC25923), Pseudomona saeruginosa (ATCC 27853), Salmonella paratyphi (ATCC 9150), Mycobacterium bovis [27290], Mycobacterium smegmatis [607] were used in this study.

Preparation of Inoculum

A loopful of the test organism (S.aureus, E.coli, P.aeruginosa, B.subtilis, K.pneumoniae and S. paratyphi) was taken from the irrespective agar slants, sub-cultured into 5mL of nutrient broth and incubated at 37°C. Following incubation at 37°C for 24 hrs, organisms were diluted with normal saline to a turbidity that was equivalent to 0.5McFarland standard (10^6CFU)/mL) (Maryam *et al*., 2018) (Woods, G, Washington J.A, Antimicrobial susceptibility test, dilution and disk diffusion methods. Manual of clinical Microbiology, 6 Ed.1995; (1327-1332) Fifty micro-litre (50 µL) of each freshly thawed stock test organism (*M. bovis and M. smegmatis*) was inoculated into 50mL of sterile Middle brook 7H9/ADC media and incubated at 37ºC with shaking for 5-7days. The activity grown *M. bovis* and *M. smegmatis* culture had its optical density adjusted to between 0.2-0.3 at a wavelength of 650nm using Jenway 6405 UV-Visible spectrophotometer.

Antimicrobial activity of samples

Stock concentrations of each sample (100 mg/mL) were tested against the test organisms using Agar well diffusion method. One hundred microliter (100 µL) of each suspension of standardized microorganisms was inoculated into sterile molten Mueller Hinton agar, swirled and poured into sterile Petri dishes and allowed to solidify. Holes for the concentration of the samples were bored aseptically using a sterile cork borer of 6 mm. The bottom of the bored holes was sealed using a drop of Mueller Hinton agar. One hundred microliters of the concentration of the sample was dispensed into appropriately labelled wells respectively. The plates were allowed to dry inside the biosafety cabinet as well as allowing the samples to diffuse for about 2 hrs and then incubated at 37°C for 24 – 48 hours. Antimicrobial activity was assessed by measuring the size of the zone of inhibition surrounding wells and taking the average of the readings of each duplicate plate post incubation (Woods and Washington, 1995).

Antimicrobial activity

The antimicrobial test of the samples was conducted using the broth micro-dilution method in 96 well microtiter plates to determine the minimum inhibitory concentration (MIC). Five hundred milligram (500 mg) of each sample was dissolved in 5 mL of sterile water to give a stock concentration of 100 mg/mL which was diluted across the 96-well micro-titre plate in a two-fold serial dilution. Each extract concentration was assayed in duplicate. About 50 µL of Mueller Hinton broth was dispensed into sterile wells of 96 microtiter plate from row 1-12. 50 µL of 100 mg/mL concentration of the samples was transferred into well 1 of the plate in duplicate. 50 µL of the solution in well 1 was transferred to well 2, mixed thoroughly and repeated through to well 11 where 50 µL was discarded. The wells (1-12) were inoculated with 50 µL of diluted organisms and incubated for 24 hours at 37°C. After the dilution procedure and inoculation, the final testing concentration was from 25 mg/mL to 0.0977 mg/mL. Post incubation, 25 µL of tetrazolium salt dye was added to all the wells, re-incubated for 1 hour and observed for absence or presence of microbial growth by colour change in the wells. The MIC was defined as the lowest drug/extract concentration that prevented the color change of the tetrazolium dye to pink. Colorless well was interpreted as no microbial growth and pink color was interpreted as growth occurrence (Jayaraman *et al*., 2008).

Antioxidant activity *Free radical scavenging assays*

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is a relatively stable free radical, which is commonly employed to determine the radical scavenging capacity of antioxidant compounds. This method relies on the reduction of DPPH in an alcohol solution in the presence of a hydrogen-donating species (antioxidant compounds) due to the formation of the non-radical form DPPH-H. This reaction produces a color change from purple to yellow, which is measured by a spectrophotometer. The disappearance of the purple color is monitored at 517 nm. The reaction mixture comprises 1.0 mL of 0.3 mM DPPH in methanol, 1.0 mL of the different concentrations of the extract (250, 125, 62.5, 31.25 and 15.625 µg/mL) and 1.0mL of methanol. It was incubated for 10 min in the dark, and then the absorbance was measured at 517nm. In this assay, the positive control was ascorbic acid (Aliyu *et al*., 2018).

3. Results and Discussion

Morinda lucida, Anogessuis leiocarpus and Sarcocephalus latifolius are reputable medicinal plants with long history of uses in Nigeria for the treatment of variety of diseases. A combination of the leaves of *Morinda lucida, Anogessuis leiocarpus* and *Sarcocephalus latifolius* prepared as a decoction is used in Nigeria as herbal remedy for treatment of *Lymphatic filariasis*, including complicated cases with wounds or sores. Extracts of the leaves of *Morinda lucida* (ML), *Anogessuis leiocarpus* (AL), *Sarcocephalus latifolius* (SL) and a combination of the three plants (Mixed) were obtained by decoction. The extracts were subjected to phytochemical analysis using standard methods and high performance liquid chromatography. The extracts were also evaluated singly and in

combination for DPPH antioxidant activity and antimicrobial effects against *Streptococcus pyogenes, Staphylococcus aureus, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella puemoniae, Bacillis subtillis, Candida albicans*.

Table 1: Extractive values of the plants singly and combination therapy

S/N	Plant	Extract Weight (g)	Yield % (w/w)
1.	*Morindalucida*	9.8273g	3.3
2.	*Anogeissusleiocarpus*	14.7117g	4.9
3.	*Sarcocephaluslatifolius*	14.3595g	4.8
4.	Combination Therapy	13.5890g	4.5

Anogeissus leiocarpus gave the highest extractive value of 4.9% (w/w), followed by *Anogeissus leiocarpus* 4.8% (w/w) and the combination therapy 4.5% (w/w) as shown in Table 1.

Table 2: Phytochemical analysis of the plants singly and combination therapy

Secondary Metabolites	Extract			
	Morinda lucida	*Anogeissus leiocarpus*	*Sarcocephalus latifolius*	Combination Therapy
Carbohydrates	Positive	Positive	Positive	Positive
Tannins	Negative	Positive	Positive	Positive
Saponins	Positive	Positive	Positive	Positive
Terpenes	Positive	Positive	Positive	Positive
Sterols	Positive	Positive	Positive	Positive
Flavonoids	Positive	Positive	Positive	Positive
Alkaloids	Negative	Negative	Negative	Negative
Anthraquinones	Negative	Negative	Negative	Negative

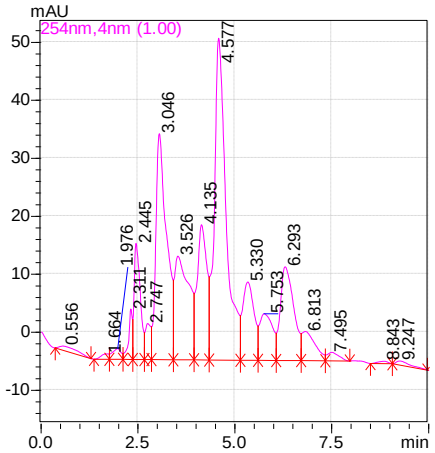

Figure 1: The HPLC spectrum of *Morinda lucida* extract.

The HPLC spectrum of *Morinda lucida* extract showed thirteen peaks with major peaks being betulinic acid (3.526 min), chlorogenic acid (4.135 min), caffeic acid (4.577 min), rutin (6.293 min) and ferulic acid (7.495 min)

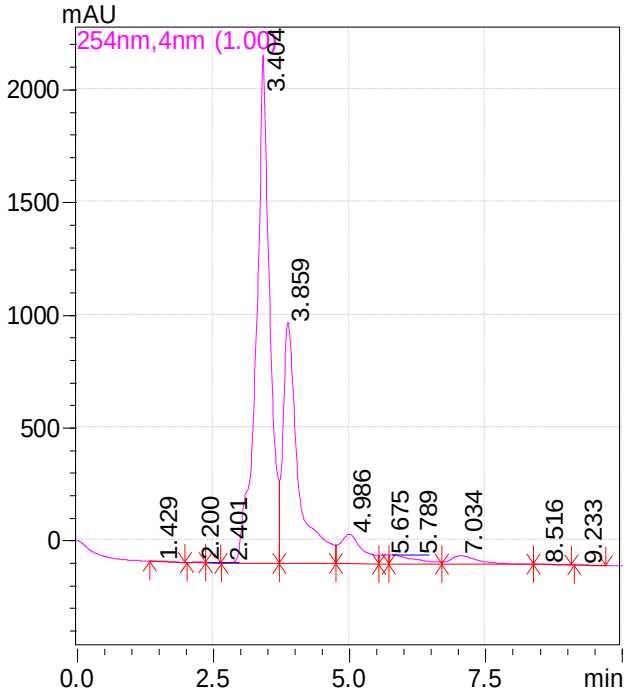

Figure 2: The HPLC spectrum of *Anogeissus leiocarpus* extract.

The HPLC spectrum of *Anogeissus leiocarpus* extract showed four peaks with major peaks being gallic acid (3.404 min), chlorogenic acid (3.859min), caffeic acid (4.986min)

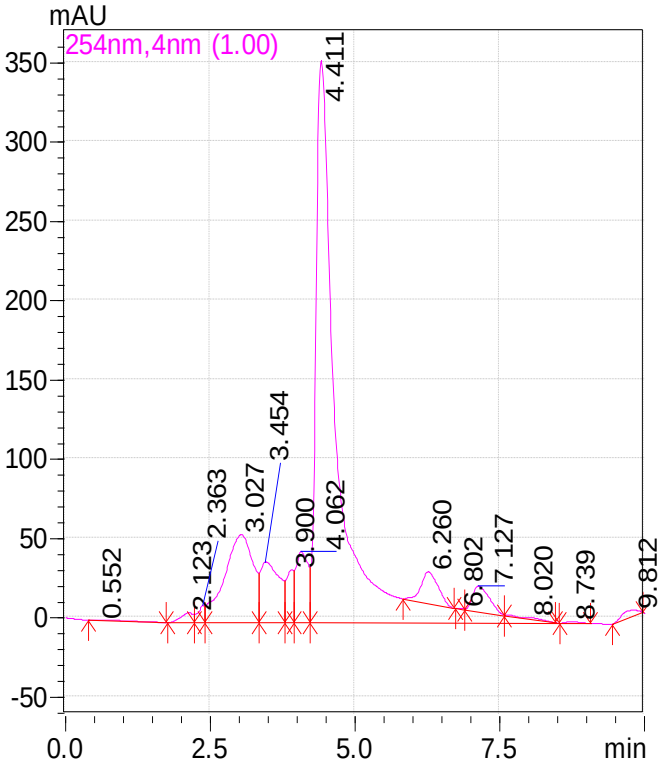

Figure 3: The HPLC spectrum of *Sarcocephalus latifolius* extract.

The HPLC spectrum of *Sarcocephalus latifolius* extract showed six peaks with major peaks being betulinic acid (2.123min), gallic acid (3.454 min), caffeic acid (4.411 min), rutin (6.260 min).

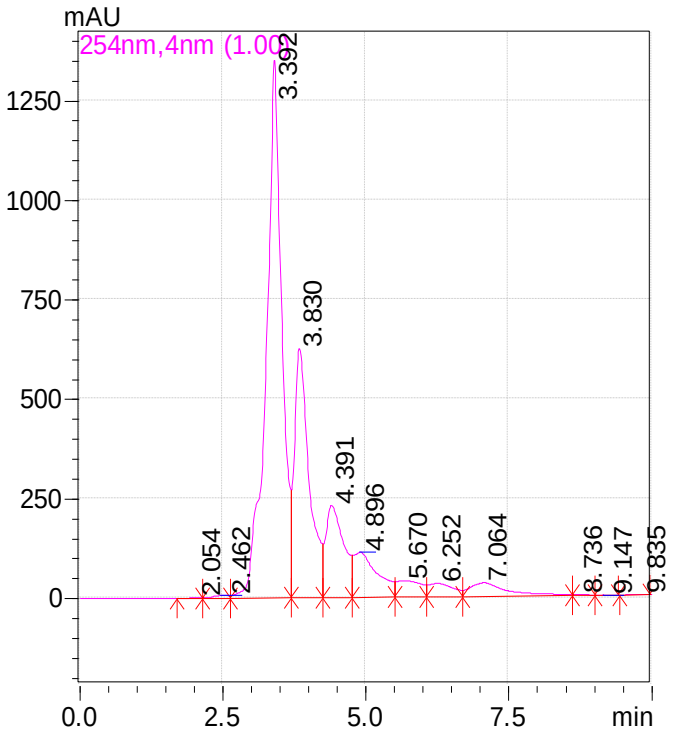

Figure 4: The HPLC spectrum of the combination therapy.

The HPLC spectrum of the combination therapy showed eight peaks with major peaks being betulinic acid (2.462 min), gallic acid (3.392 min), chlorogenic acid (3.830 min), caffeic acid (4.391 min), rutin (6.252 min)

Table 3: Antimicrobial activity of samples at 100 mg/mL concentration against microbial isolates Zone of inhibition (mm)

Extract	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Salmonella paratyphi*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella puemoniae*	*Bacillis subtillis*	*Candida albicans*
Mixed	17	17	15	10.5	13	9.5	14.5	20
AL	19	19.5	17.5	12.5	17.5	16	17	20.5
SL	9.5	-	-	-	-	-	-	9
ML	-	-	-	-	-	-	-	-

Micro organisms: *Streptococcus pyogenes, Staphylococcus aureus, Salmonella paratyphi, Escherichia coli, Pseudomonas aeruginosa, Klebsiella puemoniae, Bacillis subtillis, Candida albicans*. ML = *Morinda lucida*; SL = *Sarcocephalus latifolius*; AL = *Anogeissus leiocarpus*; MIX = Combination Therapy

Table 4: Minimum Inhibitory Concentration of samples against selected microbial isolates MIC (µg/mL)

Sample	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Salmonella paratyphi*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Klebsiella puemoniae*	*Bacillis subtillis*	*Candida albicans*
Combinations Therapy	1,560	1,560	1,560	3,125	6,250	3,125	1,560	195.3
Anogeissusleiocarpus	781.3	195.3	781.3	1,560	3125	1,560	781.3	97.7
Sarcocephaluslatifolius	6,250	NA	NA	6,250	6,250	12,500	12,500	6,250
Morinda lucida	NA	NA	NA	NA	NA	NA	NA	NA

NA= No activity

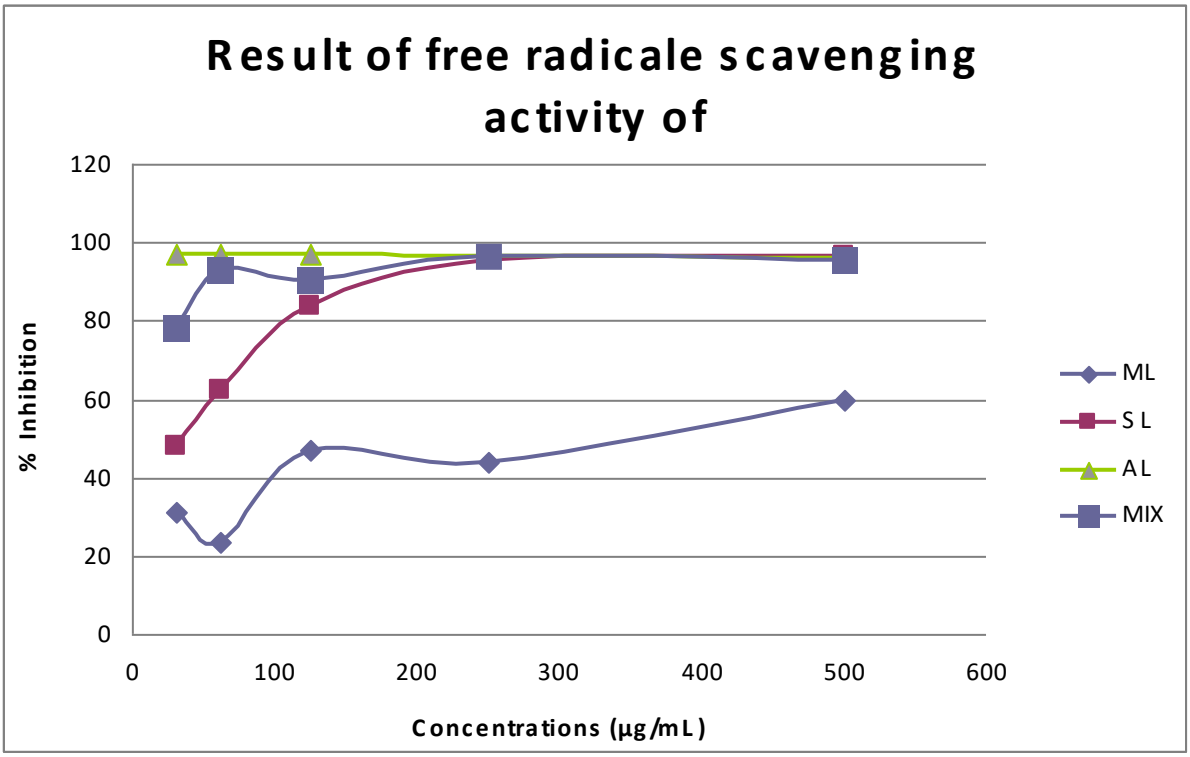

Figure 5: DPPH free radical scavenging activities of plant extracts and combination therapy.
Key: ML = *Morinda lucida*; SL = *Sarcocephalus latifolius*; AL = *Anogeissus leiocarpus*;
MIX = Combination Therapy

Anogeissus leiocarpus gave the highest extractive value of 4.9% (w/w), followed by *Anogeissus leiocarpus* 4.8% (w/w) and the combination therapy 4.5% (w/w) as shown in Table 1.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties, there are thousands known phytochemicals. It is well known that plants produce these chemicals to protect themselves from attack but recent researches demonstrate that they can protect humans against disease (Dhaarni *et al*., 2017). Some phytochemicals present in *Morinda lucida* extracts were found to be essential oils, anthraquinones, ursolic acid, oleanclic acid. The red colorants of *Morinda lucida* were confirmed to be 1-methyl-ether-alizarin, pubigdin and derivatives and lucidin, soranjodiol, damncanthol, nordammacanthal, morindin, purpuroxanthin, tannins, flavonoids, digitotolutein and saponosoides (Oludare *et al*., 2016). Some other constituents include alkaloids, saponins, glucosides, steroids, phenols, hydrogen cyanide (Adeleye *et al*., 2018). Several tests with animals confirm the attributed activity of several traditional medicinal applications of *Morinda lucida*. Extracts showed anti-inflammatory activity in test with rats and promoted gastric emptying and intestinal motility (Laval *et al*., 2012).

Phytochemical analysis showed the plants contained saponins, terpenes, sterols, phenolics and tannins. The HPLC spectrum of *Morinda lucida* showed thirteen peaks with major peaks being betullinic acid, chlorogenic acid, caffeic acid, rutin and ferulic acid. The HPLC spectrum of *Anogeissus leiocarpus* showed four peaks with major peaks being gallic acid, chlorogenic acid, caffeic acid. The HPLC spectrum of *Sarcocephalus latifolius* showed six peaks with major peaks being betulinic acid, gallic acid, caffeic acid, rutin. The HPLC spectrum of the combination showed eight peaks with major peaks being betulinic acid, gallic acid, chlorogenic acid, caffeic acid, rutin. *Anogessuis leiocarpus* extract had the highest antimicrobial and antioxidant activities followed by the combination. *Anogessuis leiocarpus* extract and the combination therapy had the highest antimicrobial (Table 4) and antioxidant activities (Figure 5). The study provided preliminary information that supports ethnopharmacological use of the plants combination therapy for treatment of *Lymphatic filariasis* with respect to antimicrobial and wound healing potential.

The effects of both aqueous and ethanolic crude extracts of stem, barks, roots and leaves of *Morinda lucida* and standard antibiotics on bacteria test showed antibacterial activity at concentration from 5 to 20mg/mol using water and ethanolic extract of the plants. All the extract exhibited antimicrobial against all tested bacteria in a concentrated dependent manner. They showed more activity against bacteria when compared with the two standard antibiotics (Chlorophenicol and liproflaxin) *Morinda lucida* demonstrated broad specimen on antibacterial and antifungal activity against *Staphylococcus aureus*, *Streptococcus pyogenus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Candida albicans*. *Morinda lucida* aqueous leaf extract on tested bacteria at different concentration 250mg/ml, 500mg/ml, 750mg/ml,1000mg/ml) showed a positive and antibacterial effect (Patience, 2008).

The phytochemical constituents present in *Sarcocephalus latifolius* includes carbohydrate, reducing sugars, tannins (not present in the fruit), saponins, flavonoids, alkaloids, steroids, glycosids, terpenoid, phenols (Yesufu *et al*., 2014). *Sarcocephalus latifolius* is among those medicinal plants widely and strongly associated with antimalarial potency. The whole part of the plant has medicinal values including the roots, barks, leaves and fruits. They are use in treatment and remedy for various ailments in humans. Its medicinal use varies from one traditional setting to another. It is used traditionally to treat fevers, pains, dental caries, septic mouth, hypertension, dysentery, diarrhea, and some central nervous system disease like epilepsy. It is an anticonvulsant, antiolytic and the root have sedative properties (Enemor and Okaka, 2013). The leaves are generally used in healing of arthritis, rheumatism, vermifuges and haemorrhoids and leprosy; the barks are medicinally used to treat blood disorders, pain killers, eye treatments, oral treatments, pulmonary troubles, skin mucosae, emetics, diarrhea, dysentery, liver diseases, genital stimulants/depressants, regulation of menstruation cycle, abortifacients, ecbolics, veneral diseases. The roots are used for treatment of stomach troubles, antiemetics, cutaneous, subcutaneous parasitic infections, fabrifuges, dropsy, swelling, oedema, gout, while the fruits are use in treatment of kidney diseases and diuretics. The fruit-juice are use to perform some religious, magic and ceremonial activities. The fruits are used as chewing sticks too (Enemor and Okaka, 2013). *Sarcocephalus latifolius* has some pharmacological activities base on their traditional uses. It has been evaluated as antiplasmodial (Udobre *et al*., 2013).

Antimicrobial activity of *Sarcocephalus latifolius* including the aerial parts (fruits, leaves, stem and bark) and roots with low activity (Ezem *et al*., 2015). Neurological effects and pain relieving activities using *Sarcocephalus latifolius.* The active principle from an analgesic fraction of the plant was recently identified as the known drug tramadol as a natural product (Boumendjel *et al*., 2013). The anti-microbial activity of crude methanol extract from *Anogeissus leicarpus* and *Terminalia avicennioides* were determined using local strains of microorganisms (*Staphylococcus aureus, Streptococcus pyogenus, Bacillus subtilis, Salmonella typhi, Escherichia coli, Candida albicans*. Combinations of the stems, barks, leaves of *Anogeissus leiocarpus* showed the highest inhibition against the test organisms (*E.coli, P.aeruginosa* and *S.aureus*) low concentration (10gml/ml) of the root extract exhibited anti bacterial activity against the organism in zone diameter studies. Combinations of roots, barks, stems bark and leave of *Anogeissus leiocarpus* on *P.aeruginosa* shows the highest diameter of inhibition (Mann *et al*., 2008).

Anti-inflammatory effect of hydro-alcoholic root and leaves extract of *Sarcocephalus latifolius* was evaluated using egg albumin-induced edema on rodent and a reduction of edema was obtained using an aqueous extract of the root bark (Abbah *et al*., 2010). The root extract possessed antidepressant, myorelaxant, antianxiety effects, decreases spontaneous motor activity and exploratory behavior, increase pentobarbital-induced sleep time and attenuates the intensity of apomorphine-induced stereotypies in mice. The leaves, roots and bark extract possess antibacterial and antiplasmodial activity due to presence of alkaloids (Taiwe *et al*., 2010).

The use of *Sarcocephalus latifolius* in treatment of dysentery and diarrhea has been reported in Ivory Coast (Ambe *et al*., 2015). Among the reported venereal disease, gonorrhrea was more specifically targeted by the genus based on traditional use of the roots and stem barks of *Sarcocephalus latifolius*, *Sarcocephalius diderrichii* and *Sarcocephalius pobeguinii* (Ambe *et al*., 2015). The genus is also used by local populations for a variety of infectious disease including scalp infections and abscess in children (Ajibesin *et al*., 2012).

Essential oils extracted from fruits of *Morinda lucida* were active on all the bacteria. The minimal inhibitory concentrations of essential oils were 32mg/ml against *P.aeruginosa* 64mg/ml against *S.aureus* and 256mg/ml on *E.coli.* The oil showed higher activity than antibiotics tested for *P.aureuginosa* (Bi *et al*., 2018).

Morinda lucida extract was proven effective in managing the dyslipdemia potentiated by alloxin, evidence in reduction of triglyceride, low density lipoprotein and total cholesterol of rats treated with *Morinda lucida*. It was noted that co-administration of *Morinda lucida* was more effective than the treatments administered singly; it was also narrowed that the result

may be attributed to the presence of flavonoids, Saponins and phenolic compound in *Morinda lucida* (Atanu *et al*., 2018).

The phytochemical composition of the *Morinda lucida* extracts indicated the presence of flavonoids, tannins and reducing sugar in all the extracts. The inhibition of alpha amylase by all the extracts at lower concentrations showed no significant difference but at higher concentration. The aqueous extract exhibited the highest inhibiting potential on the enzyme when compare to ethanol and acetone extract at concentration of 2.5-5mg/ml. The mode of inhibition of the aqueous extract of *Morinda lucida* leaf on alpha-glucoside was determined with line weaver Burk plot (Kazeem *et al*., 2013).

Aqueous stem bark extract of *Morinda lucida* strongly reduced and stretching induced by the I.P administration of acetic acid solution. Extract exhibited significant protection at 100, 200 and 400 mg/kg with maximum percentage inhibition of constriction of 80.22% observed at 400 mg/kg. The aqueous stem bark extract of *Morinda lucida* (200 and 400mg/kg) was significantly active than indomethacin 500mg/kg (Mezui *et al*., 2017).

Conclusion

The three plant extracts and the combination therapy had carbohydrates, saponins, terpenes, flavonoids, and sterols. *Anogeissus leiocarpus* had the highest antimicrobial and antioxidant activity.

This study provides preliminary evidence-based preclinical data that the combination therapy could be effective in the treatment of lymphatic filariasis (elephantiasis), subject to further clinical evaluations.

Conflict of Interest

The authors declare no conflict of interest.

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