International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685, Impact Factor: RJIF 5.28
www.pharmacyresearchjournal.com
Volume 1; Issue 4; May 2016; Page No. 05-08

Synergistic Antioxidant and Anti-bacterial Activities of *Croton zambesicus* Müll.-Arg. (Euphorbiaceae) *Leptoderris micrantha* Dunn (Fabaceae) and *Carpolobia lutea* G. Don (Polygalaceae)

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Abstract
The objective of this research was to evaluate, comparatively, the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to ascertain if there is need for the continuous use of the herbal mixture as a pro-fertility therapy. The standard methods used to investigate the antioxidant activity were the standard methods of 1, 1-diphenyl-2-picrylhydrazyl and Ferric Reducing Antioxidant Property (FRAP). Anti-bacterial activity was evaluated by the Agar well Diffusion method. The herbal mixture performed better as an anti-oxidant agent than its constituent herbal plants. As an antibacterial agent, it compared favourably with *C. zambesicus* but better than *L. micrantha* and *C. lutea.* The herbal mixture had a better antioxidant activity than any of the constituent herbal plants while *C. zambesicus* performed slightly better than the mixture and *L. micrantha* compared favourably with the mixture as anti-bacterial agents. Based on these findings, its use should be encouraged as a pro-fertility herbal therapy.

Keywords: *Croton zambesicus*, *Leptoderris micranta* and *Carpolobia lutea,* pro-fertility herbal mixture; antioxidant; anti-bacterial.

1. Introduction

The present study deals with the comparative chemical and biological properties of a pro-fertility herbal mixture and its constituent herbal plants used in South-Western Nigeria. The Yoruba tribe and their derived cultures are the main traditional inhabitants of South-Western Nigeria. Man since early times had always wanted to leave progenies behind as a means of continuity of his lineage and proof of his procreative ability [1]. Both the ability to engage in sexual trysts and consequently impregnating females culturally leaves a man with a feeling of well being and massages (boosts) his ego. The inability of a man to either have full penetrative sex or impregnate the opposite gender due to erectile dysfunction or infertility is becoming quite a common feature, and has been on the increase for some time now [2, 3]

Carpolobia lutea G. Don (family: Polygalaceae) known as cattle stick (English) and Oshunshun (Yoruba, Western Nigeria) [4] and is widely found in tropical rainforest of Africa, Guinea savannah of Sierra Leone and Cameroon as dense overgrowth or evergreen shrub of 5 m high [5]. The leaves have been acclaimed to cure malaria, snake bite, leprosy, fever, ulcer, dermal infection, venereal diseases, sterility, diarrhoea, headache and wounds. The leaves also have been acclaimed to be used to promote child birth while the root bark has been reportedly used for treating rheumatism, fever, general pain and insanity [6]. Furthermore, the decoction of the root is reputed in Western and Southern Nigeria (Yoruba ethnicity and Ibibios of Akwa Ibom State of Nigeria) as "ogun aleko" meaning sexual invigorator or aphrodisiac [7].

Leptoderris micrantha is a climbing shrub with brown stem and purple flowers. It is known locally as 'Atari Obuko' or 'Ewe Awo' in the Yoruba speaking parts of South West Nigeria. The decoction from the leaves finds use in aiding man's full penetrative ability, that is it invigorates him sexually.

Croton zambesicus is a shrub or small tree of about 16 m high found in the fringing forest and savanna, and widely distributed elsewhere in tropical Africa. The tree has a scaly bark and silvery leaves with an attractive appearance. It is often planted in towns and villages. It was planted as a fetish tree. It is believed to have a protective power, and it is often planted near the entrance of houses to ward off evil attack. Its Yoruba name, àjẹ́ kò bàlé, means 'witches do not dare to perch on it. The wood is pale yellow, fine-grained, hard and gives a good polish. The stems are used in parts of W Africa for hut-posts and in Yoruba houses for beams when other timbers are not readily available. The leaves are considered strengthening. A soup made of them is given to dysentery cases in S Nigeria. This plant has shown the presence of a trace of alkaloid in the stem and leaf [8].

2. Materials and Methods

2.1 Collection of Plant Material

Fresh plant parts, root bark of *Carpolobia lutea* (CL), stem bark of *Croton zambesicus* (CZ) and leaf of *Leptoderris micrantha* (LM) were collected from plantations in Ondo, South-west, Nigeria. Authentication was carried out by Mr. R.A. Sanni of the Department of Biology, Adeyemi College of Education, Ondo, with voucher numbers; ACH 4315, ACH 4415 and ACH 4515 respectively. These were compared with voucher specimens deposited at the Herbaria of the Department of Crop Protection and Pest Management, Federal University of Technology, Akure, Nigeria and the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. Fresh plant material was washed under running tap water, air dried, and then homogenized to fine powder and stored in airtight bottles.

2.2 Extraction of Plant Material

2.2.1 Solvent extraction

The solvent and chemicals used for this work were of analytical grade. Thoroughly washed plant parts were dried in shade for

five days and then powdered with the help of blender. The powdered plant parts were soaked in ethanol for 48 h. A brownish colour extract was obtained from CZ and CL while the extract from LM is green. CZ extract has a yield value of 8.94%, CL extract has a yield value of 3.8% while LM has a yield value of 4.2%. The solvent extracts were concentrated under reduced pressure and preserved at 5ºC in airtight bottle until further use. For the herbal mixture, 5 g of each of the air-dried powder of the herbal plant was mixed and taken in 200ml of ethanol in a conical flask, and the above procedure was repeated for its extraction.

2.3 Antioxidant Property

2.3.1 The ferric reducing antioxidant property

This was determined by assessing the ability of extracts to reduce $FeCl_3$ solution as described[9]. Briefly, extracts (0-250 µL of stock) were mixed with 250 µL 200 mM sodium phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferrocyanide, the mixture was incubated at 50ºC for 20 min, thereafter 250 µL of 10% trichloroacetic acid was added, and subsequently centrifuged at 650 rpm for 10 min, 1000 µL of the supernatant was mixed with equal volume of water and 100 µL of 0.1g/100 mL ferric chloride, the absorbance was later measured at 700 nm. A higher absorbance indicates a higher reducing power.

2.3.2 1, 1-diphenyl-2 picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2- picrylhydrazyl) free radical was evaluated as described by Halliwell *et al* [10]. Briefly, appropriate dilution of the extracts (1 mL) was mixed with 1 mL of 0.4 mM methanol solution containing DPPH (20 mg/L) free radicals, the mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Scavenging ability = A – B / A x 100

Where A is absorbance of DPPH and B is absorbance of DPPH and extract combination.

2.4 Antibacterial Activity

2.4.1 Bacterial strains

In vitro antimicrobial activity was examined for the ethanol extracts of the stem bark of the plants used by traditional healers. Microorganisms were obtained from the Department of Crop Protection and Pest Management of the Federal University of Technology, Akure, Nigeria. Among the four microorganisms investigated, one Gram-positive bacterium was *B. subtilis* while three Gram-negative bacteria were *P. aeruginosa, E. coli, and S. typhi*. All the microorganisms were maintained at 4°C on nutrient agar slants.

2.4.2 Antibacterial activity of ethanol extracts

The antibacterial activity was tested against *E. coli, S. typhi, B. subtilis*, and *P. aeruginosa* by the agar well diffusion method [11]. 24 h old Muller-Hinton broth cultures of test bacteria were aseptically swabbed on sterile Muller-Hinton agar plates. Wells of 9 mm diameter were made aseptically in the inoculated plates and the ethanol extract (20 mg/ml of 10% dimethyl sulfoxide [DMSO]), standard (streptomycin sulfate, 1 mg/ml), and control (10% DMSO) were added to the respectively labeled wells. The plates were incubated at 37°C for 24 h in an upright position. The experiment was carried out in triplicates, and the zone of inhibition was recorded.

3. Results and Discussion

3.1 Antioxidant Activity

Studies have shown that highly reactive oxygen species can be products of the low reactive oxygen species. Results of the reaction of hydrogen peroxide (H_2O_2) with low valence forms of the transition metal ions iron (Fe^{2+}) and copper (Cu^{2+}) ion are the formation of hydroxyl radical, OH, Fe^{2+}(Ferryl ion) or $Cu(OH)^{2+}$ a copper III complex. Under physiological conditions, the abundance of hydroxyl radical. OH, promotes its reactivity with any type of biological molecules in living cells, such as sugars, amino acids, phospholipids and nucleobases (the components of nucleic acids). The reducing power of substances is as a result of their antioxidant activities. As shown in Fig. 1, as the concentration of the extracts increases, the ferric reducing antioxidant property of all the samples increased. The antioxidant property of the herbal mixture is lower than those of LM, but higher than those of CL and CZ.

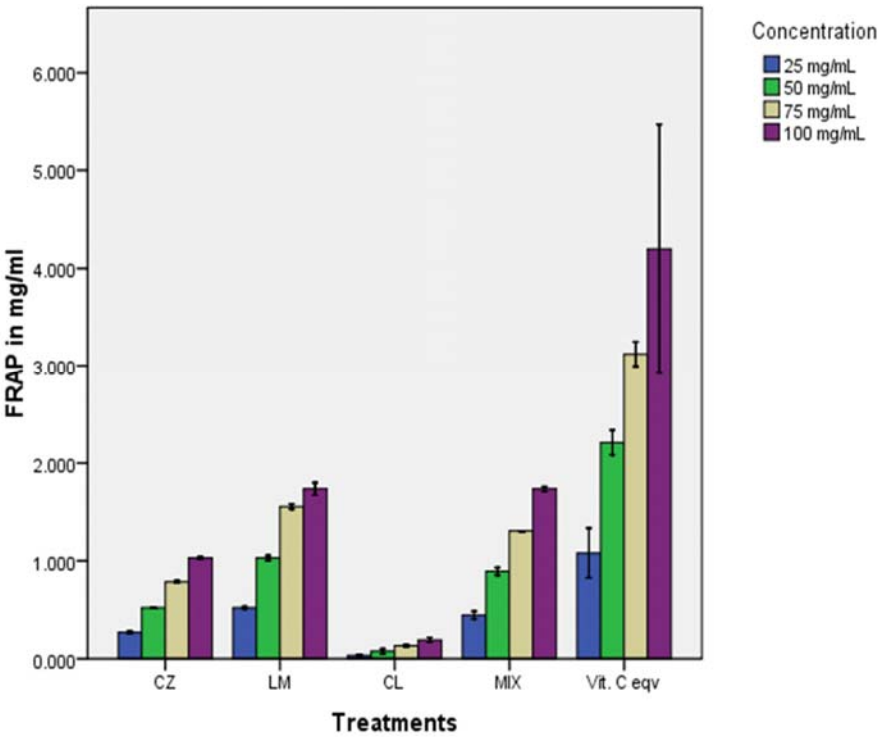
The radical scavenging activity of the extracts was observed to increase with increasing concentration. CZ had a scavenging activity of 26.7% at 50 mg/ml and 72.6% at 100 mg/ml, for LM, 21.78% was obtained at 50 mg/ml, and a maximum of 72% at 100 mg/ml. CL had 11% at 50 mg/ml, and a maximum of 63.5% at 100 mg/ml while the herbal mixture had a minimum of 7.5% at 25 mg/ml, 37% at 50 mg/ml and a maximum of 77.5% at 100 mg/ml. Instead of showing antioxidant activity, all the extracts except the MIX are pro-oxidant at 25 mg/ml. This shows that the plant extracts are toxic at low concentration of 25 mg/ml.

As the concentration of the extracts increase, the antioxidant activity of the extracts which was determined by DPPH also increases. The decrease in absorbance of DPPH is directly proportional to concentration of free radical scavenger added to DPPH reagent solution but is inversely proportional to the DPPH scavenging activity [12].

3.2 Anti-bacterial Activity

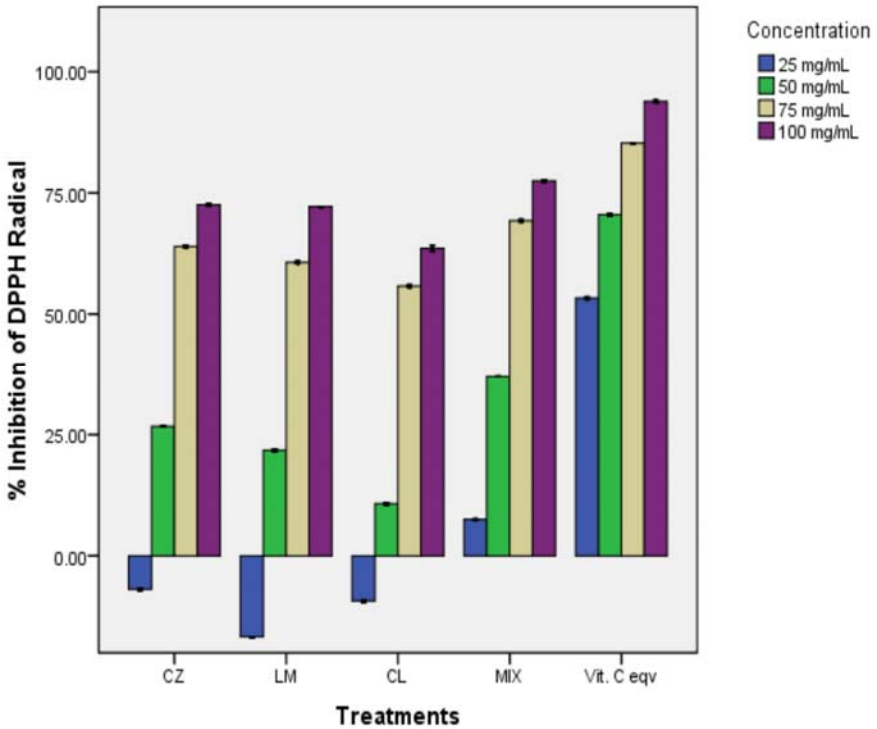
All the samples had appreciable inhibitory activity on *E. coli* with CZ and LM performing better than the MIX whereas CL did not have any inhibitory activity on it. This bacterium, *S. typhi*, was inhibited by all the extracts with CZ having the highest activity. Both LM and CL inhibited *S. typhi* equally. For *B. subtilis*, MIX had the highest activity followed by CZ. All the extracts inhibited *P. aeruginosa* appreciably with CZ having the highest activity followed by the MIX as shown in Fig. 3. Higher plants appear a good source of antimicrobials to act against microbes. Antimicrobials derived from plants are better than their synthetic counterparts because lesser side effects are noticed [13, 14]. Bio-assay guided fractionation of these extracts is required to isolate and characterize the physiologically active components having antioxidant and antibacterial activities in them.

The results indicate that the ethanolic extracts possess capabilities to neutralize the free radicals and act as an antioxidant. The bioactivities attributed to these plants may be as a result of their anti-oxidant and anti-bacterial activities. The extracts compared favourably with the standard.



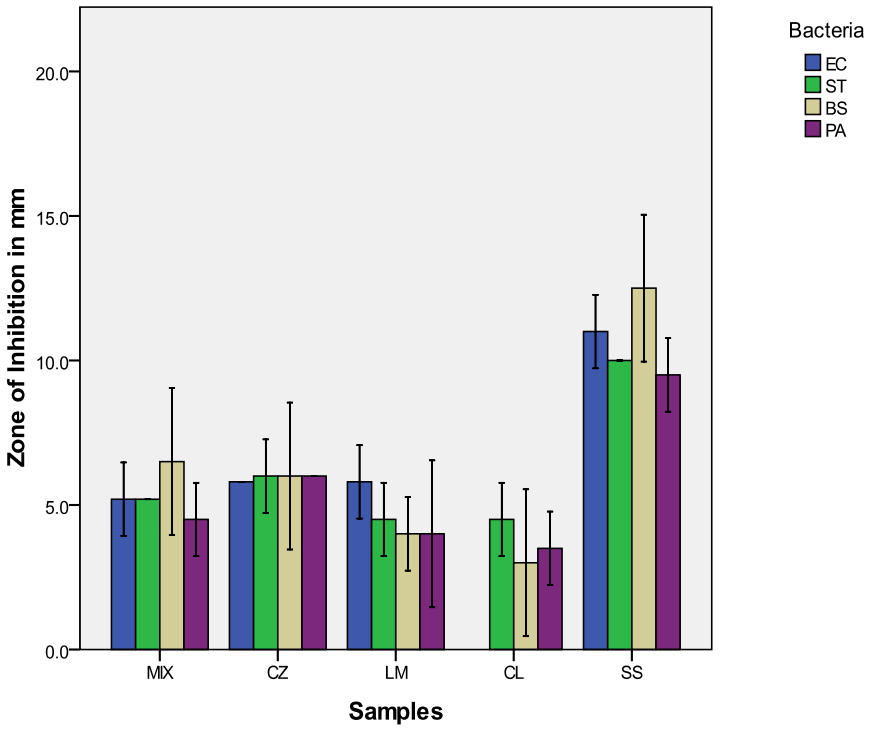
MIX= Herbal mixture; CZ=*Croton zambesicus*; LM=*Leptoderris micrantha*; CL=*Carpolobia lutea*; SS=Streptomycin sulphate

Fig 1: Ferric Reducing Antioxidant Property



MIX= Herbal mixture; CZ=*Croton zambesicus*; LM=*Leptoderris micrantha*; CL=*Carpolobia lutea*

Fig 2: Inhibition of DPPH radical.



MIX= Herbal mixture; CZ=*Croton zambesicus*; LM=*Leptoderris micrantha*; CL=*Carpolobia lutea*; SS=Streptomycin sulphate

Fig 3: Antibacterial activity of the extracts.

4. Conclusion

The herbal mixture had a better antioxidant activity than any of the constituent herbal plants while *C. zambesicus* performed slightly better than the MIX and *L. micrantha* compared favourably with the MIX as anti-bacterial agents. Based on these findings, its use should be encouraged as a pro-fertility herbal therapy.

5. References

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