

Original Research Article

**IN VITRO ANTIOXIDANT AND ANTI-BACTERIAL PROPERTIES OF A
CONTRACEPTIVE HERBAL MIXTURE OF *Z. XANTHOXYLOIDES* LAM
(RUTACEAE) *E. HIRTA* LINN (EUPHORBIACEAE) AND *A.
PRECATORIUS* L. (LEGUMINOSAE).**

ABSTRACT

Objective: Our objective was to evaluate, comparatively, the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to ascertain if the active constituents in the herbal plants are in synergy to give a better herbal therapy.

Methods: Antioxidant activity was evaluated using 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.

Results: The antioxidant assay results showed the higher antioxidant capacity of EH and ZX than the mixture and compared favourably with the positive control, ascorbic acid, while AP showed a lower antioxidant capacity than that of the mixture. The anti-bacterial activity results showed that both EH and AP have higher inhibitory activity than the herbal mixture.

Conclusion

We may, therefore, conclude that there is no scientific justification for the continuous use of this herbal mixture as a contraceptive and also as an herbal therapy for the treatment of inflammation related diseases, since both *E. hirta* and *Z. xanthoxyloides* performed better as antioxidant agents while *A. precatorius* and *E. hirta* performed better as anti-bacterial agents.

KEYWORDS: *Abrus precatorius*, *Euphorbia hirta*, *Zanthoxylum xanthoxyloides*,
Contraceptive, Herbal mixture, Antioxidant, Anti-bacterial.

1.INTRODUCTION

The present study deals with the investigation of the chemical and biological properties of a contraceptive 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. For a long time, the contraceptive methods used by men are abstinence, withdrawal, condoms, vasectomy etc. to control fertility for ages [1]. These methods have been in use until the introduction of oral contraceptives and modern intrauterine contraceptive devices (IUDs) in the second half of the 1900s. With the development of certain successful methods on females, most of the burdens of contraceptive are now on women to control population. The use of herbs is almost universal among non-industrialized societies [2]. Most Nigerians, especially those living in rural communities do not have access to orthodox medicine, and it is estimated that about 75% of the populace still prefer to solve their health problems consulting traditional healers [3].

Zanthoxylum zanthoxyloides is a prickly shrub or low-branching shrubby, very prickly tree usually growing up to 12 metres tall, occasionally to 16 metres. The bole is armed with large, woody thorns that fall as the tree grows older. It is known as “Orin ata” or “Fagara” in the Yoruba speaking area of South-Western Nigeria. The tree provides medicines, food flavourings, timber and other commodities for the local people. It is sometimes planted. The bark of the roots and stems is toxic. A dioeciously species, both male and female forms need to be grown if seed is required. The seeds are used as a pepper-like condiment. The seeds are used as beads in necklaces. Young shoots and twigs are used as chew-sticks to clean the teeth and maintain oral hygiene [4]. The bark is aromatic and sudorific. The seeds are soaked in water and used in the treatment of rheumatism [5]. The plant (part not specified) is appetizer and galactagogue [6]. The yellow wood is close-grained and extremely hard. It is durable and termite-proof [7].

Euphorbia hirta Linn. known locally in South Western Nigeria as “Emi-ile” or “Iroko Iju” and popularly called asthma weed, is one of such herbs belonging to the family *Euphorbiaceae* which is frequently seen occupying open waste spaces and grasslands, road sides, and pathways. Though a native of Central America, the herb is widely cultivated throughout the

tropics, especially in West, Central and East Africa [8]. It is usually erect, slender-stemmed, spreading up to 45 cm tall, though sometimes can be seen lying down [9]. Some of the reported phytoconstituents of the herb included triterpenoids, sterols, alkaloids, glycosides, flavonoids, tannins, phenols, choline and shikimic acid, while some of the reported scientific uses include its use as an anti-spasmodic, anti-asthmatic, expectorant, anti-catarrhal, anti-syphilitic and as a contraceptive [8;9,10].

 *Abrus precatorius* is a popular medicinal plant. It is popularly called ‘Oju Ologbo’ in South-Western part of Nigeria. Many medicinal uses are ascribed to this plant. The leaves, stem and roots are sweet-tasting due to the presence of glycyrrhizin, of which about 9–10 % is in the leaf [11]. It has been used in the treatment of coughs and vomiting in different animal species and therapeutic dose is said to be about 0.01 mg/kg body mass, above which toxicity results [12]. Children are attracted by its brightly-coloured seeds and in some countries they play with the seeds and use them in school in their handiwork and to count. Necklaces and other ornaments made from the seeds are worn by both children and adults. The seeds are also used for the treatment of diabetes mellitus and chronic nephritis [11; 13]. The plant is also used in traditional medicine for the treatment of scratches, sores, and wounds caused by dogs, cats, and mice. It is also used as a contraceptive, and with other ingredients to treat leucoderma.

In the South-Western part of Nigeria, the herbal medicine practitioners use the herbal plants being investigated here singly or as a mixture in birth control among other uses.

Hence, our objective was to evaluate, comparatively, the anti-oxidant and anti-bacteria properties of the mixture and the individual plants to ascertain if the active constituents in the herbal plants are in synergy to give a better herbal therapy.

2. MATERIALS AND METHODS

2.1 Collection of plant material

Fresh plant parts, root bark of *Zanthoxylum xanthoxyloides* (ZX), whole plant of *Euphorbia hirta* (EH) and leaf of *Abrus precatorius* (AP) 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, by comparing 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 extracted successively with ethanol in Soxhlet extractor for 48 h. A brownish colour extract was obtained from ZX while the extracts from AP and EH are green. ZX extract has a yield value of 7.94%, AP extract has a yield value of 2.8% while EH has a yield value of 7.8%. The solvent extracts were concentrated under reduced pressure and preserved at 5°C 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.

2.3Antioxidant 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 [14]. 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 50°C 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 [15]. 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.

$$\text{Scavenging ability} = A - B / A \times 100$$

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 [16]. 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 the reactive oxygen species of low reactivity can be converted to a highly reactive species. Reaction of hydrogen peroxide (H₂O₂) with low valence

forms of the transition metal ions iron (Fe^{2+}) and copper (Cu^{2+}) ion lead to the formation of $\cdot\text{OH}$ (Fenton reaction) or species of comparable reactivity such as Fe^{2+} (Ferryl ion) or $\text{Cu}(\text{OH})^{2+}$ a copper III complex. The hydroxyl radical $\cdot\text{OH}$, abundant under physiological conditions are quite reactive, reacts rapidly with any type of biological molecules in living cells, such as sugars, amino acids, phospholipids and nucleobases (the components of nucleic acids) [17]. The antioxidant activities have been reported to be the concomitant development of reducing power [18].

As shown in Figure 1, the ferric reducing antioxidant property of all the samples increased with an increase in concentration of the extracts. EH and ZX had higher antioxidant property than the mixture. But the mixture performed better than AP?

The radical scavenging activity of the extracts was observed to increase with increasing concentration. The minimum scavenging activity of 12.77% was obtained at a lower concentration of 25 mg/ml, and a maximum scavenging activity of about 79.50% was obtained at a concentration of 100 mg/ml by AP, for ZX, 32% was obtained at 50 mg/ml, and a maximum of 75%. EH had a minimum of 20% at 25 mg/ml, and a maximum of 82% at 100 mg/ml while the herbal mixture had a minimum of 26.30% at 50 mg/ml, and a maximum of 71.30% at 100 mg/ml. Conversely, both ZX and MIX are pro-oxidant at 25 mg/ml. This implies that both ZX and MIX are not safe at low concentration of 25 mg/ml. Similarly, maximum activity of 94% was also obtained from standard ascorbic acid.

The antioxidant activity of the extracts which was determined by DPPH increases with a corresponding increase in the concentration of the extracts. The decrease in absorbance of DPPH is proportional to concentration of free radical scavenger added to DPPH reagent solution. Decrease in the DPPH solution absorbance indicates an increase in the DPPH scavenging activity [19].

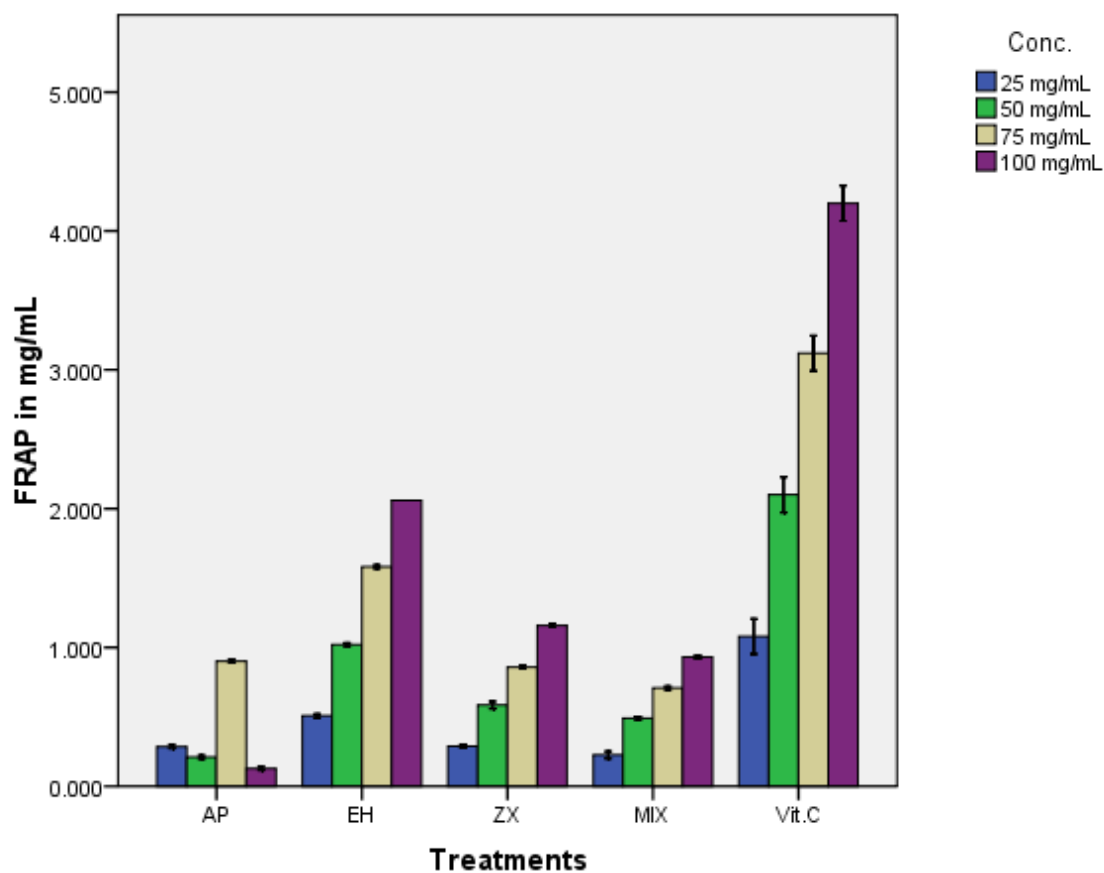


Figure 1: Ferric Reducing Antioxidant Property in mg/ml.

MIX = Herbal mixture; **AP** = *A. precatorius*; **ZX** = *Z. xanthoxyloides*; **EH** = *E. hirta*.

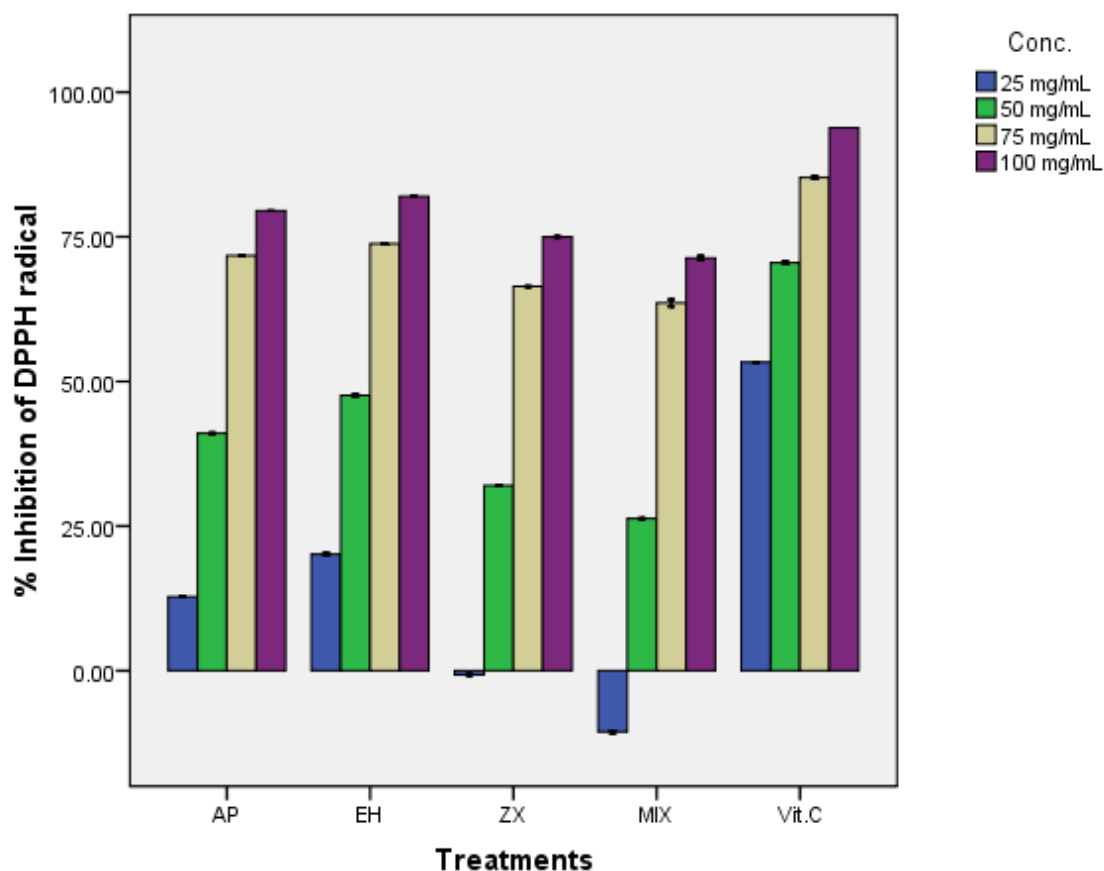


Figure 2: DPPH radical scavenging in %

MIX = Herbal mixture; **AP** = *A. precatorius*; **ZX** = *Z. xanthoxyloides*; **EH** = *E. hirta*.

The results indicate that the ethanolic extracts possess hydrogen donating capabilities and act as an antioxidant. The efficacy of these plants in some of their bioactivities may be attributed to this encouraging antioxidant potential. Though the scavenging activity of the standard was higher than those of the extracts, they are potential antioxidant drugs.

176 3.2 Anti-bacterial Activity

177 All the samples, except the herbal mixture inhibited *E. coli* appreciably with AP having the
178 highest activity while EH and ZX had the same activity. For *S. typhi*, AP also had the higher
179 activity followed by EH. This bacterium was not inhibited by both the mixture and ZX. For *B.*
180 *subtilis*, both EH and AP had the same zone of inhibition followed by the mixture, while ZX had
181 no activity against this bacterium. EH inhibited *P. aeruginosa* comparatively with the standard,
182 streptomycin, this is followed by the mixture, while ZX had the least activity (Figure 3). The
183 potential for developing antimicrobials from higher plants appears rewarding as it will lead to the
184 development of a phytomedicine to act against microbes. Plant-based antimicrobials have
185 enormous therapeutic potential as they can serve the purpose with lesser side effects that
186 incubation (zone of inhibition in mm) are often associated with synthetic antimicrobials[20].
187 Continued further exploration of plant-derived antimicrobials is needed today. Further research is
188 necessary to determine the identity of the antibacterial compounds from within these plant parts
189 and also to determine their full spectrum of efficacy. However, the present study of *In vitro*
190 antimicrobial evaluation of these plants forms a primary platform for further phytochemical and
191 pharmacological studies [21]. These extracts possess a broad spectrum of activity against a panel
192 of bacteria responsible for the most common bacterial diseases. These promissory extracts open
193 the possibility of finding new clinically effective antibacterial
194 compounds.

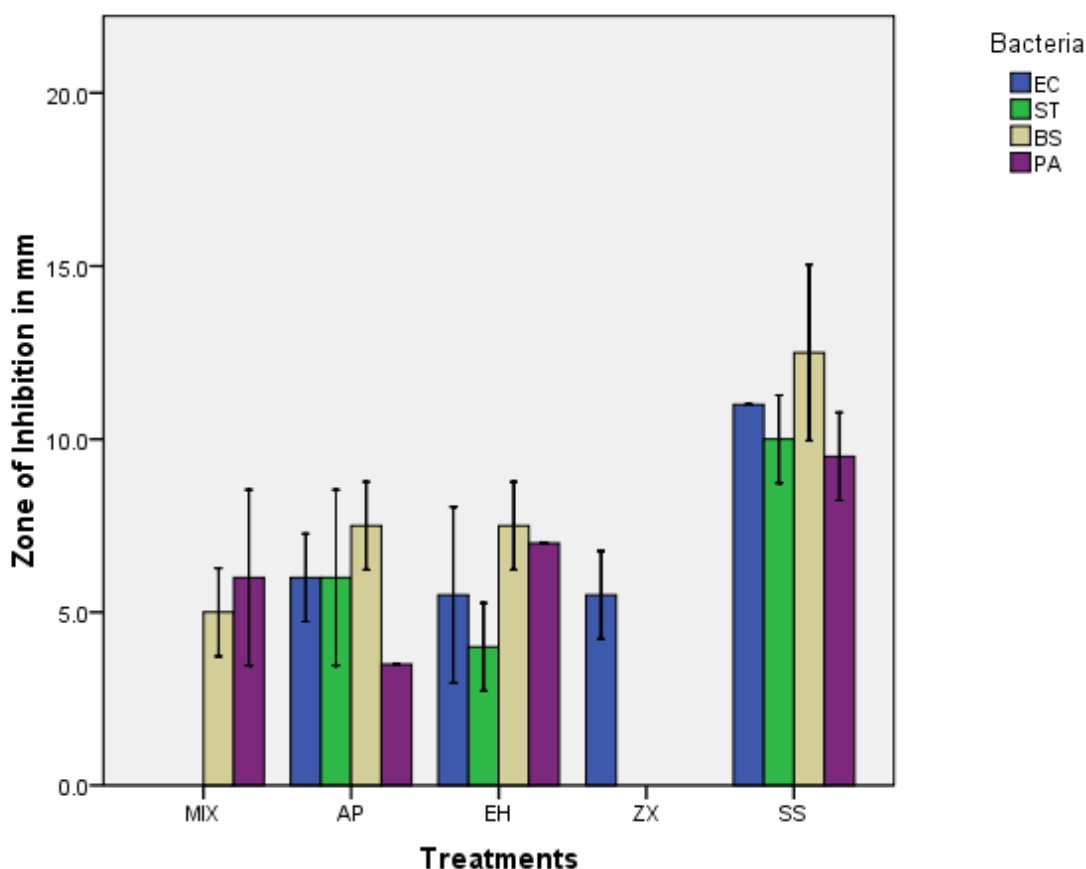


Figure 3: Anti-bacterial activity of the extracts after 24 hr of incubation.

MIX = Herbal mixture; **AP** = *A. precatorius*; **ZX** = *Z. xanthoxyloides*; **EH** = *E. hirta* ; **SS**= Streptomycin; **EC**= *E. coli*; **ST**= *S. typhi*; **BS**= *B. subtilis*; **PA**= *P. aeruginosa*

4. CONCLUSION

We may, therefore, conclude that there is no scientific justification for the continuous use of this herbal mixture as a contraceptive and also as an herbal therapy for the treatment of inflammation related diseases, since both *E. hirta* and *Z. xanthoxyloides* performed better as antioxidant agents while *A. precatorius* and *E. hirta* performed better as anti-bacterial agents.

CONSENT

It is not applicable.

206 **ETHICAL APPROVAL**

207 Not applicable

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209 **REFERENCES**

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